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A STUDY OF EGGS OF *ASCARIS LUMBRICOIDES* VAR. *SUUM* WITH THE ELECTRON MICROSCOPE*

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The egg of *Ascaris* has been widely used as a subject for cytological investigations. Nelson (1851) described the development of the egg of *Ascaris mystax.* Leuckart (1886) and Wharton (1915) reported the *Ascaris lumbricoides* egg to be surrounded by a shell and an outer, albuminous layer. The appearance of unfertilized eggs of this species was described by Otto (1932) and Keller (1933).

The number of layers surrounding the egg of ascarids has been reported as three (Nelson, 1851, *Ascaris mystax;* Leuckart, 1886, *Ascaris lumbricoides;* Ackert, 1931, *Ascaridia lineata;* Wottge, 1937, *Parascaris megalocephala;* Christenson, 1940, *Ascaris lumbricoides*), four (Kreuzer, 1953, *Ascaris lumbricoides;* Frenzen, 1954, *Ascaridia galli,* and five (Zavadovsky, 1928, *Ascaris*). A variety of techniques has been used by different authors in studying the egg layers, including microscopic observations of the penetration of substances into the egg, polarizing and fluorescent microscopy, centrifugation, and chemical tests on the membranes. The chemical composition of the layers has been investigated by several workers (Fauré-Fremiet 1913, 1913a; Chitwood 1938; Christenson, *et al.* 1942; Jacobs 1940; Timm 1950; Monné and Hönig 1954; Fairbairn 1955) and, in general, have led rather consistently to the conclusion that there are three membranes. Wottge (1937) described the endogenous formation of the egg layers of *Ascaris megalocephala,* whereas Christenson (1940) believes the outer layer of the *Ascaris* egg to be of exogenous formation.

Morita (1953) examined paraffin sections of the egg shell of *Ascaris megalocephala* with the electron microscope and concluded that the layer "consists of completely individualized microfibrils." In the present investigation, thin sections were examined with the electron microscope to determine structural details of ova and the order of formation of the egg coverings of *Ascaris lumbricoides* var. *suum.*

MATERIALS AND METHODS

Living adult *Ascaris* were removed from the intestines of freshly killed pigs and thin slices from various parts of the egg-containing uterus were placed in fixative. The eggs were fixed in either 1% osmium tetroxide according to the method of Palade (1952) for 2 hours, or Carnoy's fixative (acetic acid 20%, absolute

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alcohol 80%) for 18–24 hours. Following fixation, the ova were washed, dehydrated, and infiltrated with a mixture of 72% n-butyl- and 28% methyl-methacrylate. The eggs were sectioned at 0.025 microns with an International Minot rotary microtome, the thin sections then placed on celloidin-coated grids and examined with the aid of a model EMU–2B RCA electron microscope.

OBSERVATIONS

Organization of the Ascaris *egg.*

The egg cytoplasm of *Ascaris lumbricoides* var. *suum* is surrounded by three layers: An outer protein coat, a middle chitinous shell, and an inner lipoid lining. These layers, as observed in the living state by means of light and phase microscopes, are shown in Figure 1. The protein coat and the chitinous shell when observed in eggs removed from the uterus of the adult worm, are usually colorless and transparent. Striations are observed in the lipoid layer. Between the cytoplasm and the lipoid layer in the mature, fertilized egg is a fluid-filled cavity, the perivitelline space. Some of the egg layers and the perivitelline space are absent in unfertilized eggs (Fig. 2).

Structure of the egg by electron microscopy.

Protein Coat

The external margin of the protein layer is usually mammillated (Fig. 3). The mammillations vary greatly, appearing obtuse, undulate, or very irregular. The surface is granular, and when interrupted, the ends of fibers arising from the protein coat can be observed. In fully-formed eggs, a distinct separation between the protein and the shell layer is observed, and the protein coat terminates abruptly at the outer margin of the shell.

The protein coat appears as a very dense, reticulated material (Figs. 4 and 5) containing granules without apparent structure. The fibrils of the reticulum have a diameter of approximately 150 Angstrom units (A) and branch irregularly from the granules. The coat appears uniform in density except where it is very broad, in which case the branches are more widely separated at the outer margin.

Chitinous Shell

The chitinous shell attains a thickness of approximately three to four microns in the mature egg. The inner surface of the shell is limited by a membrane (Fig. 4) containing small granules and fibrils. The outer surface of the shell is bordered by a dense zone or membrane (Figs. 4 and 5), approximately 0.4 micron in thickness, containing granules and fibrils. At the margin of this membrane, the junction between the protein layer and the shell is marked by a narrow line (Fig. 5) approximately 200 A in thickness. The membrane is joined at intervals by the chitinous fibers of the shell. These chitinous microfibers (Fig. 5) vary in diameter from approximately 75 A to 400 A. The majority of fibers branch when reaching a length of 250 A or less to form a loose, irregular reticulum. A lamellation of the chitinous fibers was not observed.

Electron micrographs which are believed to represent successive stages in the formation of the chitinous shell are shown in Figures 6 through 11. Figure 6 shows small granular projections attached to the plasma membrane along its entire

margin. These surface projections may extend outward from the plasma membrane, or they may be elongated, attached as an inverted-U (Fig. 7), or scattered irregularly along the membrane. The projections may have been formed from the dark granules within the cytoplasm of the egg. A material which forms around the surface projections (Fig. 8), is possibly a precursor of the chitin fibers and may have been carried to the surface by cytoplasmic vacuoles (Wottge, 1937), or secreted by the projections. The chitin structure as well as the disappearance of the surface projections, is observed in Figure 9. Later stages in the process of shell formation are shown in Figures 10 and 11, where the chitinous shell has become much thicker. The plasma membrane is visible on the inner surface and the protein material is beginning to be attached on the outer surface of the shell. The embedding medium has not been removed from the latter two figures; hence the fibrous arrangement of the chitinous shell is not as immediately apparent as in Figure 9. A section through sperm is shown external to the egg in the upper part of Figure 7. All photographs in Plate III are taken at the same magnification, and the appearance of size differences is due to the part of the egg through which the section was cut.

Lipoid Layer

The lipoid layer is soluble in all common fat solvents, and therefore dissolved in the process of preparing the eggs for sectioning. The layer is not fixed by one percent osmium tetroxide or Carnoy's fixative, and the method of freeze-drying was found not to be satisfactory for its study. It was, therefore, not possible with any of these methods to obtain electron micrographs of the lipoid layer.

Perivitelline Space

The perivitelline space, which is filled with fluid as a result of "deutoplasmolysis" (Lams, 1952), appears optically empty; no structures of any kind were observed with the methods of fixation used.

Cytoplasm

Large numbers of granules and vacuoles are located within a very coarse cytoplasmic reticulum (Figs. 12 and 13). The vacuoles, varying in size from 0.3 to 1.7 microns in diameter, are optically empty; a fine, fringed border is sometimes seen surrounding the inside of the wall. The vacuoles may be concentrated in the central portion, or scattered at random in the cytoplasm.

The granules of the cytoplasm, called "brown granules" by Boveri (1910), vary in diameter from 0.1 to 0.8 microns. No structural details were observed and whether or not some of the granules are mitochondria is uncertain.

In the immature egg, large, homogeneous, ovoid structures called "hyaline spheres" by van Beneden (1883) were observed (Fig. 13). They vary in diameter from 1 to 4 microns and are enclosed by a granular membrane. At the outer margin of some immature eggs, material which appears similar to that contained within the spheres was observed. The hyaline spheres are dissolved in acetic solutions (Fauré-Fremiet *et al.*, 1953, 1954) and were observed only with osmium tetroxide fixation.

The nucleus, or pronucleus (Fig. 14), approximately four microns in diameter, is surrounded by a granular membrane. It contains dark bodies, presumably chromatin material, as well as a loose protoplasmic reticulum.

DISCUSSION

The nature of the egg envelope and therefore a knowledge of its ultrastructure are of interest. The controversy which exists concerning the number of membranes in nematode eggs was summarized by Jacobs (1940) who indicated, "the difference in the number of membranes described by various authors can . . . be attributed to the variety of techniques used in studying the eggs".

The electron micrographs presented herein support the belief that there are three layers surrounding the egg cytoplasm of *Ascaris lumbricoides* var. *suum*, viz. the outer protein coat, the middle chitinous shell, and the inner lipoid layer. The report of Frenzen (1954) that the egg envelope of *Ascaris lumbricoides* includes four layers is not supported by the present work. The portion of the egg indicated as a separate layer by him is probably the zone of separation between the protein coat and the chitinous shell. This zone, although more dense, is not believed to represent a distinct egg layer, since close observation at high magnification reveals a typical chitin structure. Furthermore, the report of Zavadovsky (1928) that the egg cytoplasm of *Ascaris* is enclosed by five layers is not supported by the present investigation, since a lamellation of the chitinous shell was never observed.

Jaskoski (1952) suggested that the outer protein coat represented an important auxiliary barrier against the passage of materials in or out of the ascarid egg. Germans (1954) concluded that the "slime layer" confers protection against desiccation. Wottge (1937) reported that the protein coat contained a lipoid substance, and this material might be located in the interstitial vacuoles of the protein reticulum. The network arrangement of the protein coat with interposed lipoid material, as well as the limiting membranes separating the egg layers, may lend support to these conclusions.

The endogenous formation of the protein coat of the egg of *Ascaris megalocephala*, advanced by Wottge (1937) was based on the presence of a protein covering around unfertilized eggs. Chitwood (1938), however found eggs without this layer and suggested that the coat was a uterine secretion added after the two inner layers had formed. In support of this latter point of view, Lowry *et al.*, (1941), observed granules within the cytoplasm of the uterine cells of *Ascaris equorum*, and suggested that the granules represented an albuminous substance which is secreted into the uterus, and surrounds the eggs as they pass along it. The electron micrographs of the pig *Ascaris* egg fail to show the protein coat surrounding either the oocyte as described by Wottge (1937), or the outer margin of those eggs in which the chitinous shell was in the process of formation. In view of these observations, the order of formation of the egg envelope is believed to occur as follows: the chitinous shell is formed first, followed by the appearance of the lipoid lining, both of these layers of endogenous formation; the protein layer of the egg is added last as a product of uterine secretion.

Kreuzer (1954) suggested that the chitinous shell provides mechanical protection to the egg and preserves stability of form. Wottge (1937) referred to this layer as the "homogeneous membrane" because of the clearness and transparency of the layer, and Schmidt (1936) indicated that the shell substance was homogeneous and composed of non-fibrillar material. Observations of the chitinous shell with the electron microscope indicate that the shell is distinctly fibrillar. The suggestion of Monné and Hönig (1954) that the shell of *Ascaris* consists of chitin

fibers and protein lamellae which alternate with each other seems not to be warranted on the basis of these observations. The photograph published by Morita (1953) as a cross-section through the shell, actually appears to be a photograph of the outer protein layer. The size of the chitin fibers, as reported by this author, probably represents the measurements of the protein strands of the outer coat, and consequently the lower limits are much too large. In addition, it is believed that the cross-section of the shell, in any case, would not have the appearance pictured.

Monné and Hönig (1954) indicated that the lipoid coat is the portion of the egg envelope which protects the egg against penetration by harmful chemicals, and Kreuzer (1954) suggested that this layer produces a completely effective diffusion barrier to ions. The ultrastructure of this region will be of interest when fixation techniques for the study of this material are devised.

Fauré-Fremiet, *et al.* (1953, 1954) reported that the hyaline spheres were distributed at random in the cytoplasm of the oocyte, and passed to the periphery after fertilization, where they coalesced and disappeared into the perivitelline space. The role and significance of the spheres remain undetermined.

SUMMARY

1. Studies of ova of *Ascaris lumbricoides* var. *suum* with the electron microscope support the belief that the egg envelope is composed of three layers which in order of formation include a middle chitinous shell, an inner lipoid layer, and an outer protein layer. The protein coat and the chitinous shell are bordered by limiting membranes.

2. The protein coat of the egg, whose margin may be smooth or mammillated, appears as a dense, reticulated material, with fibrils having a diameter of about 150 A.

3. The chitinous shell is composed of highly branched microfibers which vary in diameter from approximately 75 A to 400 A. A lamellation or orientation of the chitin fibers in any one plane of the egg was not observed. Electron micrographs, which may represent successive stages in the formation of the chitinous shell, are presented.

4. The lipoid layer, in the living state, has a striated appearance. A suitable method for the fixation of the layer, however, was not found.

5. Electron micrographs fail to reveal structures of any kind in the perivitelline space of the egg.

6. Large numbers of granules and vacuoles are located within a very coarse cytoplasmic reticulum inside the egg itself. The hyaline spheres, observed in immature eggs, are described.

7. The highly branched arrangement of the protein coat and the chitinous shell, as well as the limiting membranes separating the egg layers, may help explain the extreme resistance of *Ascaris* ova to many environmental changes.

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Explanation of Plates

Plate I

Fig 1. Living fertilized egg. P protein coat, S chitinous shell, L lipoid layer. ×1500

Fig. 2. Living unfertilized egg. P protein coat. ×700

Fig. 3. Fertilized egg. P protein coat, S chitinous shell, C cytoplasm. Carnoy's fixative. Embedding medium not removed. ×12,000

Plate II

Fig. 4. Mature egg. P protein coat, S chitinous shell. Carnoy's fixative. ×25,600

Fig. 5. The fibrous structure of the protein coat P and the chitinous shell S are shown. Osmium tetroxide fixation. ×64,000

Plate III

Figs. 6–11. Successive stages in the formation of the chitinous shell. Figures 6–7, Osmium tetroxide fixation. Figures 8–11 Carnoy's fixative. Embedding medium not removed in figures 10–11. All figures ×12,000.

Plate IV

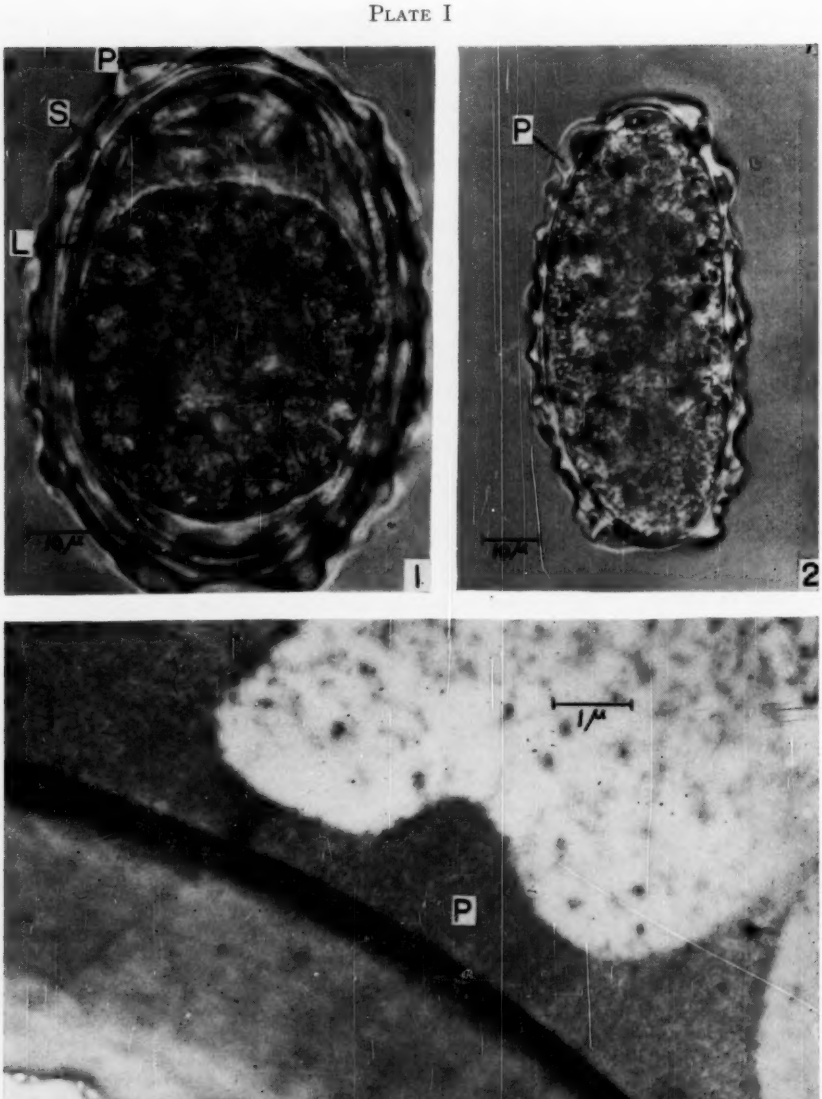
Fig. 12. Mature egg. P protein coat, S shell, N nucleus. Carnoy's fixative. ×7200

Plate V

Fig. 13. Cytoplasm of an immature egg in which the egg envelope has not yet formed. H hyaline sphere, V vacuole, G brown granule. Osmium tetroxide fixation. ×12,000

Fig. 14. Nucleus of egg enclosed by granular membrane. N nucleus. Carnoy's fixative. ×18,400

PLATE I



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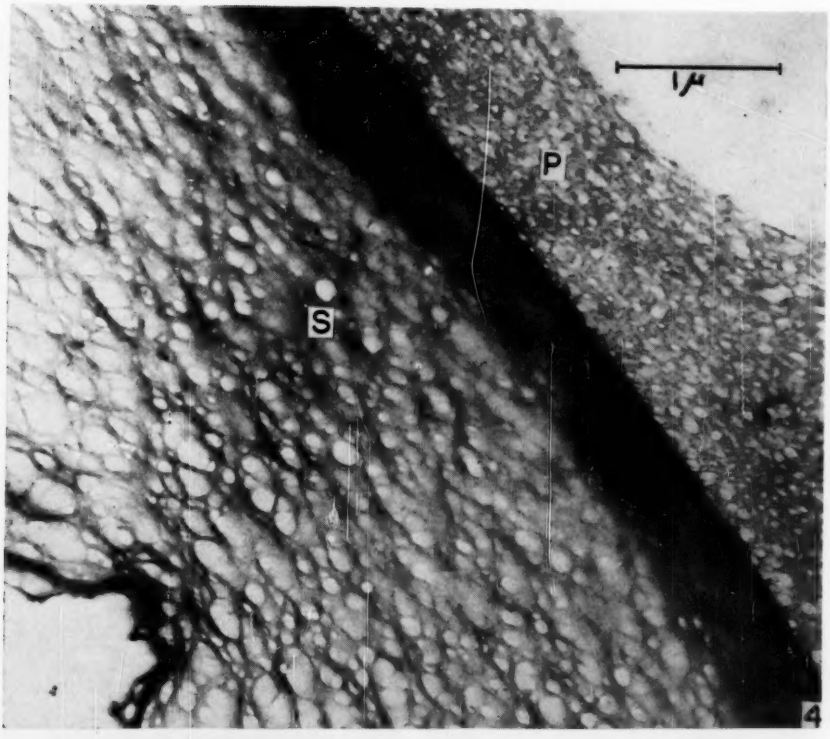

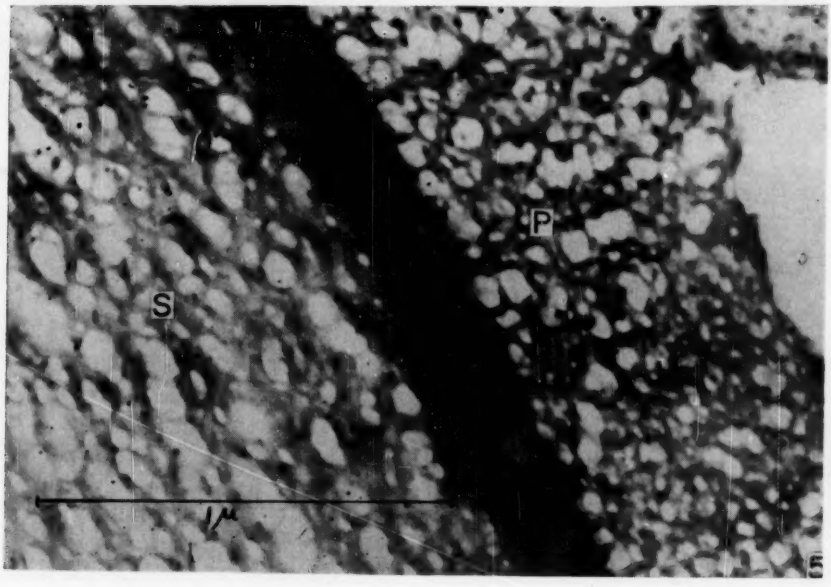

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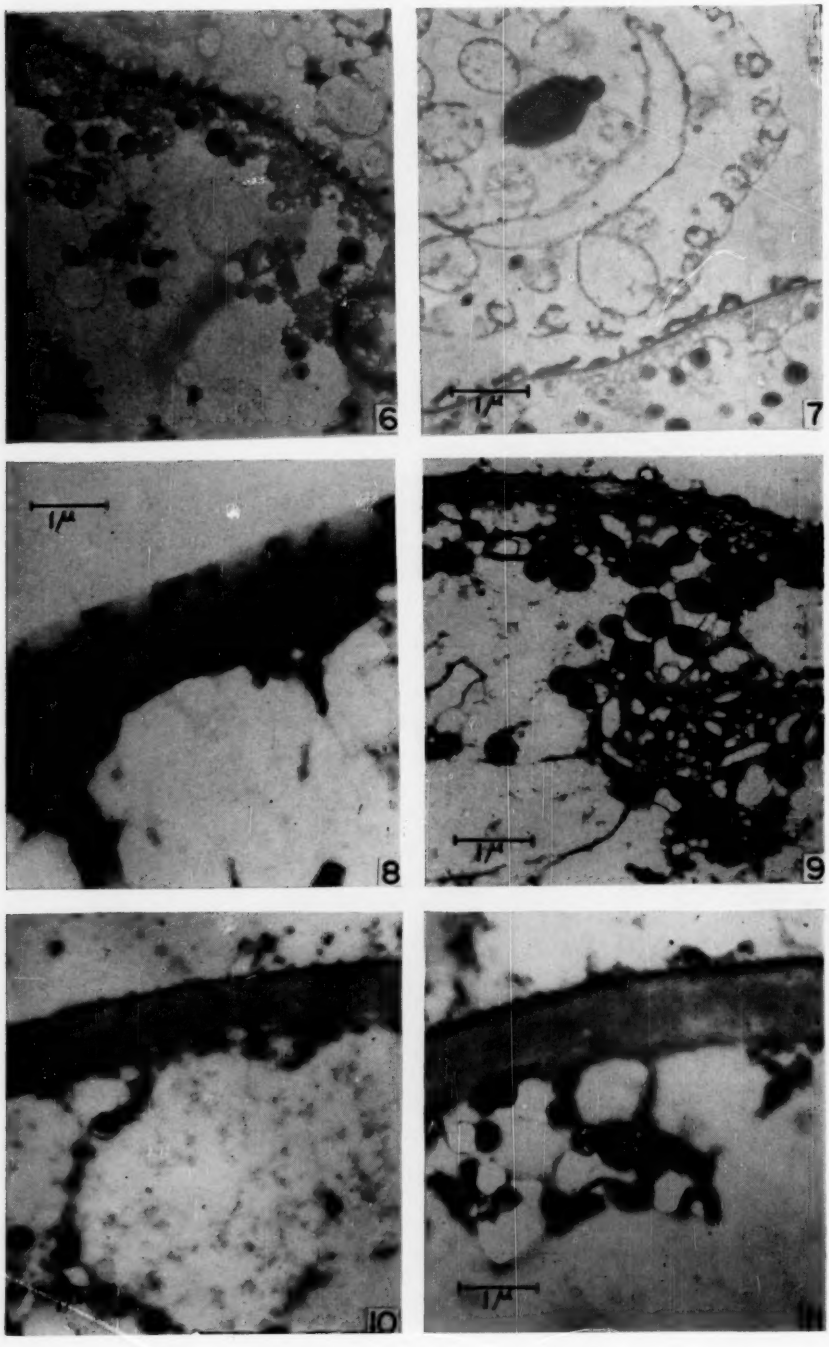
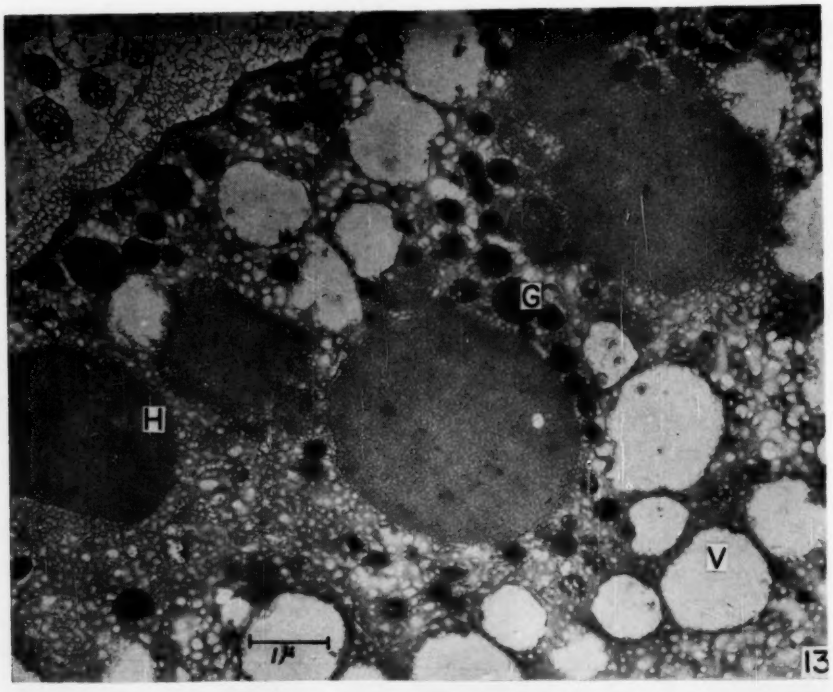
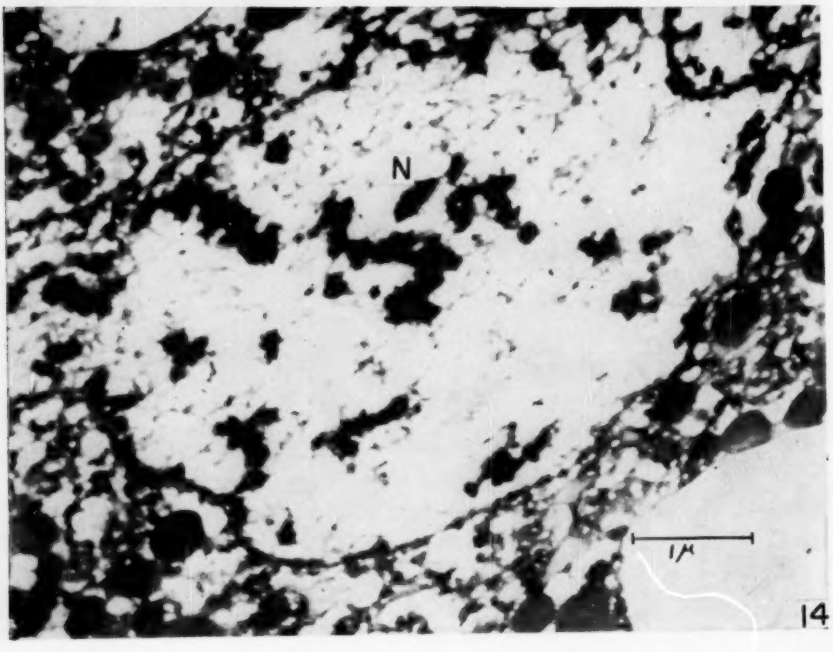


PLATE IV

PLATE V





AN ELECTROPHORETIC STUDY OF SERA FROM RATS ARTIFICIALLY INFECTED WITH AND IMMUNIZED AGAINST THE LARVAL CESTODE *CYSTICERCUS FASCIOLARIS**

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INTRODUCTION

Studies on *Cysticercus fasciolaris* infection have made significant contributions to our knowledge of immunity against parasites. Conclusive demonstrations of acquired (Miller, 1931b), artificial (Miller, 1931a) and passive (Miller and Gardiner, 1932) immunity against a metazoan parasite emanated from these efforts. Miller and his group showed: (1) infected rats were immune to a superimposed infection, (2) intraperitoneal injections of worm material induced immunity and (3) protection could be passively transferred.

Campbell (1938a, b, c) demonstrated an "early" and "late" immunity involving at least two antibody mechanisms. The former destroys the larvae prior to encystment in the liver, is present in the serum collected on the 11th day after infection and is absorbable with homologous worm material. Late immunity is evidenced following encystment, is present in serum collected 28 days after infection and is non-absorbable. The "early immunity" is probably exerted against the larval somatic antigens and the "late immunity" may involve a response against metabolic products and/or essential metabolites of the parasite.

It is likely that the basic mechanisms operative in the immune response against the animal parasites are essentially the same as those functioning against other infectious agents (Culbertson, 1951). Electrophoresis proved a valuable tool in immunological studies of other fields and has not been adequately applied to the problems of immunity to animal parasites. In Stauber's (1954) review of the applications of electrophoresis to parasitology, he has noted the failure to demonstrate conclusively in which serum protein fraction(s) antibodies against animal parasites are found. Such information would contribute much to our understanding of the relation between the basic immune response against animal parasites and other infectious agents.

The purpose of the study herein reported was twofold. First, it was desired to ascertain the effects of a *C. fasciolaris* infection in rats as reflected in the protein metabolism. As this parasite usually localizes in the liver with a resultant liver tissue involvement (Bullock and Curtis, 1924, 1926), any alterations in liver function would likely be manifested in the rat's serum protein metabolism. Secondly, an attempt was made to relate the rat's humoral response against the parasite to the

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serum proteins. By analyses of sera from infected and artificially immunized rats, it was hoped to determine in which protein fraction(s) the antibodies appeared.

MATERIALS AND METHODS

General Procedures:

Cats were used for maintaining a source of *Taenia taeniaeformis* eggs. All cats, upon arrival in the laboratory, were immunized against feline distemper with feline distemper vaccine (Pitman-Moore Company, Indianapolis, Indiana).

Rats used in an experiment were of the Wistar or Sprague-Dawley strains. Males were employed throughout because of the greater resistance of females to an infection with *Cysticercus fasciolaris* (Campbell and Melcher, 1940). The rats were kept in a room completely isolated from all other laboratory animals. Food (Miller's Eaties, Battle Creek Dog Food Company, Battle Creek, Michigan) and water were available *ad libitum*.

Several factors such as age (Heim and Schechtman, 1954), sex (Moore, 1948) and plasmapheresis (Chow *et al.*, 1948) induce significant variations in the serum electrophoretic pattern of an animal. These were obviated as much as possible when serum was acquired for electrophoretic analysis by having all littermates segregated as equally as possible into control and experimental groups. Food was withdrawn the evening prior to the day of bleeding from the animals to be bled.

Blood was obtained by maximally bleeding each rat, under sodium pentobarbital anesthesia supplemented by ether, by intracardiac puncture under aseptic conditions and then discarding the rat. After clotting at room temperature overnight, the serum was collected and clarified by centrifugation for 30 minutes at about 2,000 rpm. The clarified serum was analyzed immediately or, when necessary, stored at 4°C. for not longer than 48 hours before analysis. To insure an adequate amount of serum for testing, sera collected from 2–3 rats per experimental and control group were pooled.

Eggs used for infecting rats were obtained by sacrificing an infected cat on the day rats were to be infected. The gravid segments of the recovered worms were teased apart and the eggs recovered. Quantification of the eggs was accomplished by using a hemacytometer. Rats were infected intragastrically by introducing one ml. of the evenly dispersed egg suspension (in 0.85% sodium chloride) through a No. 8 French catheter that was orally passed into the stomach.

Whole cysticerci were used as antigen for artificial immunization. They were stored at minus 30° C., ground into a paste with a mortar and pestle and made into a 10 or 20 percent suspension in saline (0.85% sodium chloride) for use in the intraperitoneal injections of the rats being immunized. For the sera absorption studies fresh larvae and lyophilized larvae and adults were used as the homologous antigen. Campbell's (1938b) method was employed for serum absorption, adding enough antigen to the serum to make a final 2% suspension.

Immune (from infected or artificially immunized rats) and control sera for passive immunization studies were obtained by pooling the sera from 8–10 rats per group. Each rat received intraperitoneally 1 or 2 ml. of serum, depending upon amount of serum available, within 24 hours prior to or following their being infected with *Taenia taeniaeformis* eggs.

The number of larvae present in the livers of infected rats was determined by

counting the total number of cysts visible on the surface of the entire liver. All animals were routinely necropsied and any found harboring cysts from an accidental infection were discarded.

Total Protein Determinations:

Serum total protein was determined by the biuret reaction, using Weichselbaum's (1946) biuret reagent. A standard curve was calibrated using a crystalline bovine albumin solution (Armour and Co., Chicago) with a specified nitrogen content (about 10 mg. of protein nitrogen per ml.) as the standard protein solution. The standard solution was converted into grams percent of protein by multiplying the nitrogen content by the factor 6.25. Readings were made on a photelometer at 525 millimicrons.

Electrophoresis:

All sera were analyzed by the moving boundary electrophoresis technique using the Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus. Barbiturate (veronal and sodium veronal) buffer of pH 8.6 and 0.1 ionic strength was used throughout this study.

Sera were diluted with buffer 1:2, generally 2 ml. serum and 4 ml. buffer. Dialysis was performed by the mechanical stirring method of Reiner and Fenichel (1948) at room temperature with the diluted serum enclosed in a membrane of seamless regenerated viscose process cellulose (Visking Corp., Chicago). Dialysis of the diluted sera was effected against 400 ml. of buffer for 1 hour followed by 600 ml. of fresh buffer for 4 hours.

The 2 ml. capacity cell was used and the following average particulars employed: 7.5 milliamperes at 112 volts for 7600 seconds; potential gradient of 8.18 v/cm; bath temperature of 0.5° C.

Ascending and descending limbs were photographed by the scanning method (Longsworth, 1946). All calculations were made from two-fold enlargement tracings of the descending limb patterns. The base line was established with the aid of a scanning photograph of the cell limb taken prior to shifting the initial boundary into view (Longsworth and MacInnes, 1940). Area measurements were made by delineating each area according to the method of Tiselius and Kabat (1939); then, the total area (exclusive of the epsilon-boundary) and component areas were determined with a planimeter. By appropriate calculations, each serum protein component was obtained in terms of relative percent composition and grams percent protein. Mobilities were obtained by using the equation given by Longsworth and MacInnes (1940).

Experimental Protocols:

Experiment I. Eight-week-old Wistar strain rats were each infected with 7,000 eggs. Bleedings of infected and corresponding littermate controls were made at 4-day intervals thereafter up to 28 days. A final bleeding was made on the 35th day.

To test by the *in vivo* method the protective capacity of the sera collected on the 35th day, 13-week-old rats were separated into 3 groups with littermates distributed as equally as possible. Within 24 hours after being infected with 1,200 eggs, each rat of one group received intraperitoneally 2 ml. of normal rat serum,

the second group 2 ml. of infected rat serum, and the third group no serum. They were all autopsied 5 weeks after infection.

Experiment II. This experiment was essentially a repeat of Experiment I and was undertaken in an effort to establish in which serum protein fraction(s) the "early" and "late" immune factors are present. Eight-week-old Sprague-Dawley rats were each infected with 3,000 eggs. Sera were collected from normal and infected rats on the 4th, 8th and 12th day after infection. The 12th day sera were absorbed with fresh larval paste and reanalyzed electrophoretically.

To correlate any changes in the electrophoretic patterns with the protective capacity of the sera, the latter were tested by passive immunization. Distributing littermates as equally as possible, 5 groups of 10-week-old rats were set up as follows: 1. received no serum; 2. received normal rat serum; 3. received normal-absorbed rat serum; 4. received infected rat serum; 5. received infected-absorbed rat serum. Each rat received one ml. of serum intraperitoneally and was then infected within 24 hours with 3,000 eggs. Necropsy was performed 4 weeks after infection.

The procedure outlined for the 12 day bleeding was repeated on the 35th day. Details differed only in the use of lyophilized larvae for sera absorption, a new lot of eggs (dosage of 3,500) and rats 13 weeks and 4 days old for passive immunization.

Experiment III. In order to circumvent the changes in the serum electrophoretic patterns of infected rats induced by liver damage, this study was undertaken. Sprague-Dawley rats were artificially immunized by a series of 6 intraperitoneal injections of a 10% suspension of ground larval paste. The injections (1 ml./injection) were initiated when the rats were 8 weeks old and were given on alternate days with a rest period between the 3rd and 4th injections. A final injection of a 20% suspension was given 4 days prior to the last bleeding.

Immunized and control rats were bled at 4 day intervals following the first injection up to the 28th day. Sera collected on the 28th day were utilized for absorption studies. The procedure outlined in Experiment II was followed. Lyophilized adults were used for absorbing the sera.

For the passive immunization study, rats 12 weeks and 4 days old, were infected with 1,500 eggs within 24 hours after receiving the serum (1 ml. intraperitoneally). They were autopsied 4 weeks after infection.

RESULTS

Experiments I and II:

Results of the sera analyses of Experiment I are presented in Table I. All of the electrophoretic data, as expressed in terms of both grams percent protein and relative percent composition, the total proteins and A/G (Albumin: Total Globulin) ratios obtained in this study were analyzed statistically. The "t" value was determined for the mean of the differences between the control and experimental groups. "t" values at or beyond the 5% and the 1% levels of probability were considered significant and highly significant, respectively. An examination of Table I shows that the differences occurring in the total protein and alpha 1-globulin were not significant. The gamma-globulin of the infected group was significantly increased. Total globulin, alpha 2-globulin and beta-globulin of the

TABLE I. *Electrophoretic analysis, Experiment I*

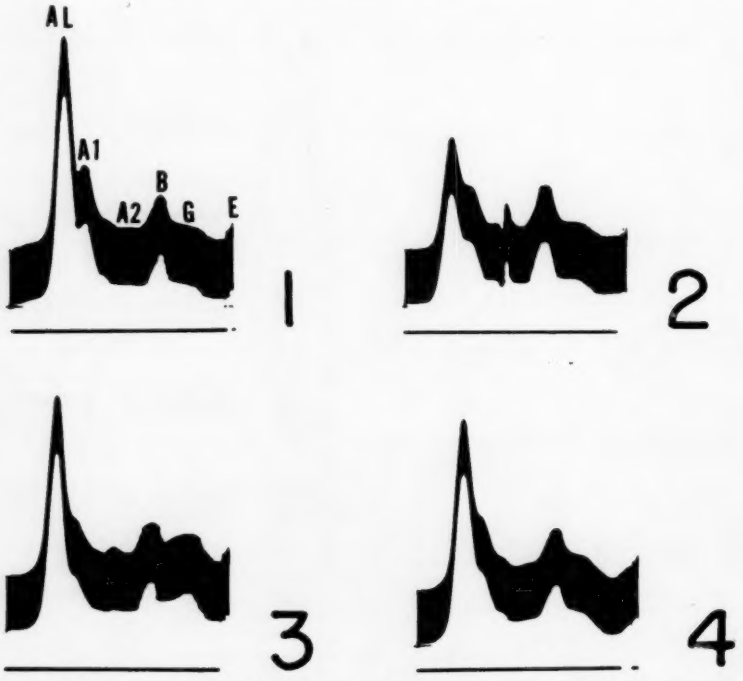
No. rats used	Age (Days) bled	Days Since Infected	Total Protein (Gm. %)	Electrophoresis (Gm. %)						
				Albumin	Total Globulin	Globulins				A/G Ratio
						Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control Group:										
1	60	–	5.89	3.19	2.70	.84	.52	.78	.56	1.18
1	64	–	5.47	2.78	2.69	.87	.51	.88	.43	1.03
2	68	–	5.43	3.02	2.41	.81	.41	.76	.43	1.25
2	72	–	5.22	2.91	2.31	.84	.45	.75	.27	1.26
1	76	–	5.75	3.27	2.48	.94	.45	.77	.32	1.32
2	80	–	6.19	3.62	2.57	.71	.54	.92	.40	1.41
2	84	–	6.41	3.47	2.94	1.19	.51	.94	.30	1.18
6	91	–	6.66	3.58	3.08	1.17	.59	.91	.41	1.16
		Average	5.88	3.23	2.65	.92	.50	.84	.39	1.22
Infected Group:										
3	60	4	6.05	3.08	2.97	.82	.72	.88	.55	1.04
1	64	8	5.73	2.11	3.62	.92	.74	1.45	.51	.58
2	68	12	6.03	2.88	3.15	.81	.44	.90	1.00	.91
2	72	16	5.45	2.93	2.52	.60	.52	.90	.50	1.16
2	76	20	5.75	2.88	2.87	.65	.64	1.09	.49	1.00
2	80	24	6.21	3.30	2.91	.77	.63	1.02	.49	1.13
2	84	28	6.19	3.03	3.16	.87	.64	1.14	.51	.96
9	91	35	6.79	3.16	3.63	.93	.75	1.34	.61	.87
		Average	6.02	2.92	3.10	.80	.64	1.09	.58	.96
Level of Significance			None	Exceeds 1%	Exceeds 1%	None	Exceeds 1%	Exceeds 1%	Exceeds 5%	Exceeds 1%

TABLE IV—*Electrophoretic analysis, Experiment III*

No. rats used	Age (Days) bled	Days since first injection	No. of injections received	Total Protein (Gm. %)	Electrophoresis (Gm. %)						
					Albumin	Total Globulin	Globulins				A/G Ratio
							Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control Group:											
3	60	-	-	5.90	3.15	2.75	.97	.66	.75	.37	1.15
3	64	-	-	6.06	3.05	3.01	.88	.53	1.19	.41	1.01
3	68	-	-	6.29	3.26	3.03	.99	.67	1.04	.33	1.08
3	72	-	-	5.50	2.84	2.66	1.10	.46	.79	.31	1.07
2	76	-	-	5.77	3.00	2.77	1.06	.46	.89	.36	1.08
2	80	-	-	6.48	3.35	3.13	1.14	.53	.97	.49	1.07
9	84	-	-	6.13	3.14	2.99	1.08	.57	.91	.43	1.05
			Average	6.02	3.11	2.91	1.03	.55	.93	.39	1.07
Immunized Group:											
3	60	4	2	6.21	3.38	2.83	1.00	.62	.91	.30	1.19
3	64	8	3	6.06	2.94	3.12	1.02	.64	1.08	.38	.94
3	68	12	5	6.19	3.02	3.17	1.11	.64	.94	.48	.95
3	72	16	6	5.65	2.77	2.88	1.02	.56	.87	.43	.96
2	76	20	6	6.10	3.16	2.94	1.12	.49	.99	.34	1.07
2	80	24	6	6.34	3.25	3.09	1.14	.50	.95	.50	1.05
10	84	28	7	5.98	2.78	3.20	1.08	.54	.97	.61	.87
			Average	6.08	3.04	3.03	1.07	.57	.96	.43	1.00
		Level of Significance		None	None	None	None	None	None	None	None

infected animals showed significant increases; the albumin and A/G ratio had decreased significantly.

Of particular interest are the marked changes that occurred on the 8th, 12th and 16th days after infection. On the 4th day, the only noticeable change was the increased alpha 2-globulin of the infected group; alpha 2-globulin persisted at a fairly constant level above the control group throughout the experimental period (Table I). A sharp increase in the beta- and total globulins and marked decrease in the albumin and A/G ratio appeared in the infected group on the 8th day (Figures 2 and 5). By the 12th day the beta- and total globulins dropped sharply to



FIGS. 1 to 4. Sera electrophoretic patterns (descending limb) of Experiment I. 1, normal; 2, 8th day after infection; 3, 12th day after infection; 4, 35th day after infection (AL, albumin; A1, alpha 1-globulin; A2, alpha 2-globulin; B, beta-globulin; G, gamma-globulin; E, epsilon-boundary.

levels still above those of the controls while the albumin and A/G ratio rose markedly to values still below those of the uninfected rats. The albumin was near the control value on the 12th day, had essentially the same value as the controls on the 16th day and thereafter maintained itself below the control albumin level (Figure 5). A sharp rise in the gamma-globulin of the infected rats was seen on the 12th day and abruptly returned to a lower level above that of the controls on the 16th day (Figures 3 and 5).

From Table I and Figure 5 it can be seen that the differences between the significantly altered serum proteins of the control and infected rats from the 16th day on were relatively constant and are still apparent in the serum pattern of the

35th day (Figure 4). It appeared that the protein metabolism was most drastically affected for approximately two weeks (between the 4th and 16th days) after infection.

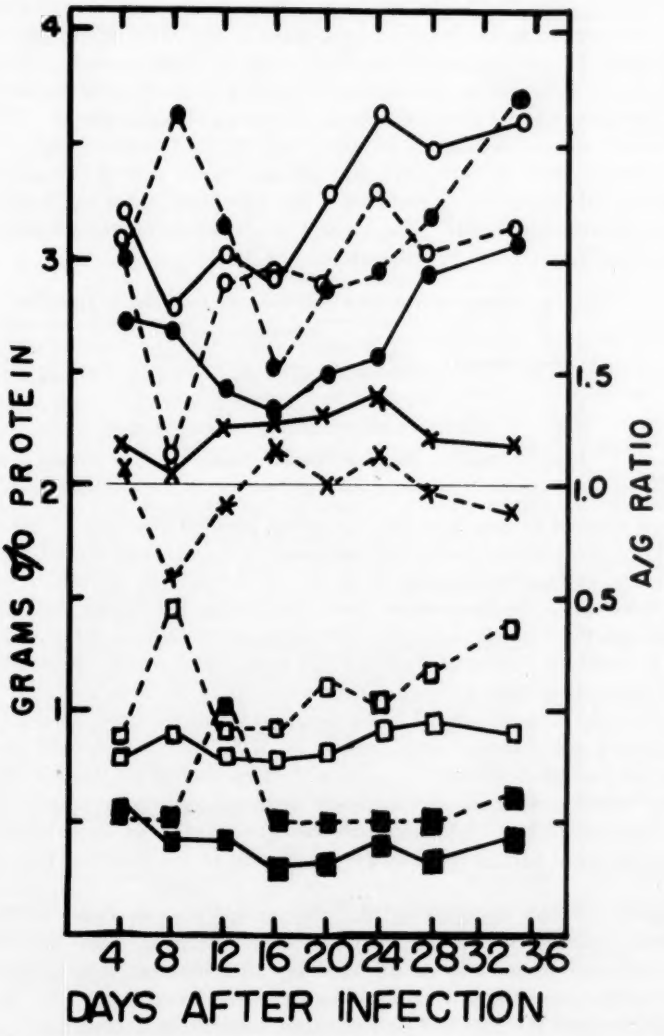


FIG. 5. Relationship of total globulin, albumin, A/G ratio, beta- and gamma-globulins to time of infection. Total globulin, ●; albumin, O; A/G ratio, X; beta-globulin, □; gamma-globulin, ■; control, ———; infected, - - - -.

Qualitative changes in any serum protein component as manifested by the appearance of new components or alterations in their mobilities were not observed in any of the studies herein reported. However, qualitative changes as evidenced by the presence of a protective humoral factor did occur. Rats passively immun-

ized with the sera of infected rats, collected on the 35th day, were completely protected against infection.

Experiment II was an effort to demonstrate in which serum protein fractions(s) the antibody against the larvae appeared. Though comparison of Experiments I and II was limited by the fewer analyses made in the latter, the results obtained in Experiment II were in general similar to those of Experiment I. The data of Experiment II were not as quantitatively significant as those of Experiment I. Total protein and alpha 1-globulin were again not significantly altered. Total and beta-globulins showed significant increases and the A/G ratio a significant decrease in the infected animals. Whereas albumin, alpha 2- and gamma-globulins were significantly altered in Experiment I, the differences noted in Experiment II were not statistically adequate. The average results of the electrophoretic analyses of Experiment II are given in Table II.

TABLE II. *Average values for electrophoretic analysis, Experiment II.*

Group	Total Protein (Gm.%)	Electrophoresis (Gm. %)						
		Albumin	Total Globulin	Globulins				A/G Ratio
				Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control	5.64	2.91	2.73	1.01	.54	.81	.37	1.08
Infected	5.66	2.66	3.00	.93	.59	1.02	.46	.90
Level of Significance	None	None	Exceeds 5%	None	None	5%	None	Exceeds 5%

It is of interest to note here that the abrupt changes observed on the 8th and 12th days of Experiment I were also apparent in Experiment II, but to a lesser degree. The explanation appears to lie in the fewer cysts present in the rats bled on these days. In Experiment I rats bled on the 8th day had an average of 160 cysts and those on the 12th day 225 cysts. Rats bled on the corresponding days of Experiment II averaged 66 and 124 cysts, respectively. Furthermore, in a preliminary experiment extending over a period of 35 days the total average number of cysts present was 27. No significant changes were obtained in any of the protein components, total protein or A/G ratio. However, qualitative changes were assumed to have occurred as rats re-infected on the 35th day after the initial infection were almost completely protected against the second infection. Hence, it is evident that the quantitative changes obtained in Experiments I and II are for the most part, if not entirely, a reflection of the extent of liver involvement.

Campbell (1938b) demonstrated that the "early" immune factor is absorbable from serum collected on the 11th day following infection. Sera collected from infected and non-infected rats on the 12th day after infection were analyzed electrophoretically before and after absorption with fresh larval paste. No significant alterations between the pre- and post-absorbed patterns were obtained.

That the sera collected on the 12th day contained protective humoral bodies was evident from the results of the passive immunization study. Also, some absorption of antibodies from the sera was accomplished though it appeared to be somewhat inadequate and possibly accounts for the failure to induce any change in the electrophoretic pattern (Table III).

Negative results were also obtained in the absorption studies conducted on the 35th day sera. Antibodies were also present in the 35th day serum of the infected

rats as both pre- and post-absorbed sera afforded complete protection to passively protected rats (Table III). Failure to obtain evidence of any absorption of anti-

TABLE III—*Average number of larvae in livers of rats passively immunized with serum collected from rats on the 12th and 35th days, Experiment II*

Day serum collected	Rat serum received				
	None	Normal	Normal-absorbed	Immunized	Immunized-absorbed
	Cysts	Cysts	Cysts	Cysts	Cysts
	Living - Dead	Living - Dead	Living - Dead	Living - Dead	Living - Dead
12th*	251.7 - 46.7	171.0 - 20.0	144.7 - 33.0	0 - 11.0	0 - 45.7
35th**	12.0 - 99.0	6.8 - 31.3	0.75 - 43.0	0 - 0	0 - 0

*Each group contained 3 rats
**Each group contained 4 rats

body in the rats receiving the post-absorbed serum is in agreement with the non-absorbability characteristic of the "late" immune factor with worm material (Campbell 1938b, c).

Though the "late" immune factor was not absorbable by the method employed, it was felt that it might be possible to relate changes hoped for in the absorbed 12- and 35-day sera. Thereby, evidence may have presented itself enabling the establishment of the serum protein component(s) in which the "early" immune antibody is produced with a possible extension to the "late" immune factor.

Experiment III:

Antibody induced with larval worm material is absorbable (Campbell, 1938b), and it seemed that this afforded an excellent approach to determining the serum protein component in which the "early" immune factor is produced; and also enabled the elimination of changes superimposed by liver involvement in infected rats.

Table IV presents the results of the electrophoretic analyses of this experiment. No statistically significant changes were obtained.

Electrophoretic patterns of the pre- and post-absorbed sera collected on the 28th day showed no significant alterations in any of the protein components. Immune sera showed protective capacity (Table V.) The qualitative changes might

TABLE V—*Average number of larvae in livers of rats passively immunized with serum collected from rats on 28th day of Experiment III*

Rat serum received				
None* Cysts	Normal Cysts	Normal - absorbed Cysts	Immunized Cysts	Immunized-absorbed Cysts
Living - Dead	Living - Dead	Living - Dead	Living - Dead	Living - Dead
19.0 - 58.5	22.5 - 49.3	19.3 - 34.0	2.0 - 17.0	11.3 - 35

*Each group contained 4 rats

be due to antibodies produced in response to immunization. Also, absorption had been moderately successful in spite of the failure of the pre- and post-absorbed sera patterns to indicate any significant changes. This may also be a reflection of inadequate absorption.

DISCUSSION

The serum protein changes noted in the infected rats cannot be regarded as specific. If anything, the significant quantitative differences noted are attributable essentially to the liver tissue involvement resulting from the infection. Hypo-

albuminemia and hyperglobulinemia are characteristic of many conditions including parasitic infectons (Stauber, 1954) and liver involvement (Gutman, 1948). Significant decreases in the A/G ratios reflected the rise in total globulins and decreased albumin though the total protein was unaffected. Failure of the total protein to indicate quantitative alterations in serum protein components is not unusual in liver conditions (Gutman, *loc. cit.*).

Among the globulins, gamma-globulin is most frequently increased in liver involvement with changes in other globulins varying with different liver conditions (Gutman, 1948). The significant increments in the alpha 2-, beta- and gamma-globulins noted in the heavily infected animals are essentially what would be expected in a condition of liver involvement. Also, the severity of the condition (i.e., number of larvae in the liver) influences the degree of serum protein changes (Gray and Barron, 1943).

Evidence indicates that the liver is the primary site of albumin synthesis (Madden and Whipple, 1940; Peters and Anfinsen, 1950) and that albumin production is impaired with a resultant hypoalbuminemia when liver parenchyma is damaged (Martin, 1949). Other studies suggest that alpha 1-globulin is formed largely in the liver (Roberts and White, 1949) and that globulins in general are mainly produced in extrahepatic sites (Abrams and Cohen, 1949).

That hyperglobulinemia is a physiological compensation for the decreased albumin in order to maintain the blood's colloidal osmotic pressure is highly questionable (Gutman, 1948). Several factors have been incriminated as responsible for the globulin disturbances in liver diseases. Gamma-globulin changes have been attributed to liver damage (Lamirande, 1952) and liver mesenchymal cell activity (Popper *et al.*, 1951; Franklin *et al.*, 1951). Alterations in the alpha-globulins are considered to reflect liver damage (Popper *et al., loc. cit.*), proliferative activity (Lamirande, 1952) and possibly a non-specific response to tissue destruction in the case of alpha 2-globulin (Seibert *et al.*, 1947). Liver proliferative activity may be concerned in beta-globulin responses (Lamirande, 1952).

From the above, the serum protein changes noted in the infected rats appear correlated with the liver tissue reaction to the infection described by Bullock and Curtis (1924). For approximatly 8–10 days following infection, liver cell degeneration predominates. The marked decrease in albumin found on the 8th day is indicative of impaired liver function as a result of the liver damage. On the 12th and 16th days albumin approached or was at the control level and coincided with the time liver cells were rapidly recovering. As albumin persisted below normal values thereafter, it is felt that the traumatic effects occasioned by the growth in size of the larvae and their cyst wall on the liver cells were moderately adverse to albumin synthesis. Though not statistically significant, the lowered alpha 1-globulin in the infected rats may also be related to liver damage.

A proliferative stage involving liver mesenchymal cells starts about 8–10 days after infection and succeeds the degenerative phase. By the 12th day the proliferative stage is well underway and liver parenchyma cells also show active division. The marked increment in beta-globulin noted on the 8th day corresponded to the beginning of the proliferative stage. Continuance of an elevated beta-globulin throughout the period studied probably reflects proliferative activity in the liver. Maintenance of alpha 2-globulin increases in the infected rats would appear to be

due to proliferative activity and also in part a nonspecific response to liver tissue damage as on the 4th day (during degenerative phase) it was the only globulin showing a definite elevation. Gamma-globulin manifested an abrupt rise on the 12th day at which time proliferative activity is well underway. As mesenchymal cell activity is then prominent, the gamma-globulin increase may be associated with enhanced mesenchymal tissue activity and/or be a response on their part to the products of liver cell degeneration that accumulated during the first week.

Proliferative activity begins to subside on about the 20th day after infection, but never entirely ceases. The abrupt changes in the serum proteins were encountered during that period following infection (4–16th days) when liver tissue involvement was most pronounced. Protein metabolism appeared to have been markedly altered within this time. Following this period, concentration differences between infected and control rats were relatively stable. As liver tissue activity began subsiding at this time, it would seem that the protein metabolism had readjusted to an atypical and fairly steady state of activity.

The highest concentrations of beta- and gamma-globulins occurred about the time that the absorbable "early" immune factor is present (Campbell, 1938b). However, as a consequence of the negative results obtained in the absorption studies it is only possible to conjecture as to which protein component contained the humoral factor. The gamma-globulin might be favored, but it is to be borne in mind that antibody production is not restricted to any one protein component (Enders, 1944). Hence, in spite of the quantitative and qualitative changes noted it is only feasible at this time to infer therefrom that the alterations were primarily non-specific results engendered in the liver involvement. Other electrophoretic studies of parasitic infections have failed to delineate the specific and non-specific changes in the protein components (Wright and Oliver-Gonzalez, 1943; Leland, Lindquist and Lillevik, 1955).

Though the artificial immunization work eliminated the superimposition of liver damage effects, no evidence presented itself as to the protein component containing the "early" immune factor. There were no significant quantitative differences giving portent as to the localization of the qualitative changes exemplified in the passive immunization study. The absorption studies were also fruitless.

Several factors may enter into the failures encountered. The absorption methods used were empirical (Campbell, 1938b) and serve to emphasize the need in parasitic immunology of more exact quantitative techniques for antigen-antibody studies. Inability of the electrophoretic apparatus to detect the antibody produced and absorbed as well as inadequate absorption are to be considered. Immunizing materials and procedures used in addition to the bleeding schedule may enter into the situation.

Further immunization studies employing cyst-fluid material for determining the serum protein component site of the "late" immune factor, isolated protein fractions and the immunoelectrophoretic techniques (Williams and Grabar, 1955) could prove fruitful.

Electrophoretic studies of serum proteins serve as indicators of the physiological state of the animal. Investigations of this type would not only contribute to our understanding of antibody production in parasitic infections but also to the problem of the host's physiological response to parasitism. This, in turn, should facilitate elucidation of the overall host-parasite relationship complex.

SUMMARY

Sera from rats experimentally infected with *Cysticercus fasciolaris* were analyzed electrophoretically for various days following infection. A similar procedure was carried out on sera from rats artificially immunized with larval worm material. This is believed to be the first application of electrophoresis to the study of a cestode infection.

New components or alterations in the component mobilities were not obtained. However, qualitative changes in the serum proteins of infected and artificially immunized rats were evident from the positive results in passive immunization and reinfection studies.

In the infected rats the quantitative changes in serum protein components are related to the degree of infection. Rats having less than 66 cysts exhibited no significant quantitative changes. Significant quantitative increases in heavily infected rats occurred in the total, alpha 2, beta- and gamma globulins. Albumin and the A/G ratio were significantly decreased. Rats containing fewer larvae than the heavily infected rats showed significant differences only in the total globulin, beta-globulin and A/G ratio.

The apparent correlation of the serum protein changes with alterations in serum protein metabolism resulting from the liver tissue reaction against the infection is discussed. It is concluded that the serum protein changes were, for the most part, a non-specific response manifesting liver involvement.

Animals were artificially immunized in order to circumvent the changes superimposed by the liver response in the hope of determining the protein component containing the "early" immune factor. Significant quantitative differences were not obtained though immunity was produced.

Results of the efforts to ascertain the immunologically active protein component in infected and artificially immunized rats by electrophoretic analysis of serum before and after absorption with worm material were negative.

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IMPORTANCE OF THE EGG SHELL OF *ASCARIDIA GALLI* TO THE INFECTIVITY OF ITS LARVA*

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Infections with *Ascaridia galli* may be conditioned by the age, diet, breed, and sex of the fowl (Ackert *et al.*, 1928, 1927, 1933, and Sadun, 1951). The present study was concerned with other possible extra-host factors which could condition the degree as well as the incidence of infection of fowls with this roundworm. Guberlet (1924) and Ackert (1931) reported that hatched infective larvae, when given orally, were rarely infective to chicks. This work suggested to the present authors that an investigation of the role of the egg shell surrounding the larva might be a factor conditioning infection of the definitive host. A preliminary report of this work was made by Hansen and Turner (1954).

MATERIALS AND METHODS

All chickens used in the experiments were White Rocks obtained from a commercial hatchery. They were raised in brooders and later housed in growing-batteries until termination of the experiments. The chicks were fed a standard commercial ration.

Eggs of *A. galli* were obtained and cultured using a modified method of Hansen *et al.*, (1954). This modification involved pipetting off the artificial digestive juice after the eggs had settled to the bottom of the Petri dish. The eggs were then washed with three changes of tap water, which was carefully pipetted off after each change. If the artificial digestive juice has been washed away, formalin is not necessary in the cultures to prevent mold growth. This modification in technique greatly reduced the time for preparation of egg cultures. The eggs and hatched larvae used in each experiment were from a single culture.

At 13 days of age the chicks were banded and divided into six groups of nearly equal weights according to the method of Gardiner and Wehr (1950). These groups were established on the basis of the nature of eggs and hatched larvae given to them and the route of administration. There were 10 chicks per group in Experiment I and 20 per group in Experiments II and III. Each of the 14-day-old chicks in all groups received 50 ± 5 embryonated eggs or hatched larvae. Three groups received eggs or larvae orally by means of a pipette; Group CO received normal (control) embryonated eggs, Group TO received eggs chemically treated with a special solution to dissolve the shells, and Group HO was given hatched larvae. The same type of de-shelled eggs or hatched larvae also were injected into the duodenum of three additional groups of chicks, designated as Groups CD, TD, and HD, respectively. The group letters serve to identify not only a specific group but also to show the kind of eggs or larvae and the manner of administering them. All groups were sacrificed 21 days after infection, and all worms were recovered from the small intestine and counted.

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The solution used to de-shell the ova consisted of equal parts of 3% sodium hydroxide and a 3% solution of commercial sodium hypochlorite (5.25% NaOCl) as described by Elliott (1954). The eggs used for infecting chickens in the T-groups and for obtaining hatched larvae for the H-groups were exposed to this solution for 24 hours. Subsequently, they were washed 4 or 5 times in tap water to remove all traces of the treating solution. The eggs used for the H-group chicks were shaken vigorously in a shell vial containing about a half teaspoon of fine sand. This technique facilitated the hatching of large numbers of larvae. Pitts (1948) reported that a sodium hypochlorite-sodium hydroxide solution dissolved the shells of *Ascaris* eggs in 24 hours leaving the embryos surrounded only by thin elastic shell membranes. He also observed that motile embryos produced bulges in the elastic envelopes, and in many instances were liberated by their own activity. Similar observations were made in the present study using sodium hypochlorite-sodium hydroxide treated *Ascaridia* eggs. By use of a fine glass needle in a micromanipulator, the vitelline membrane of the de-shelled eggs (Jacobs, 1940) was found to be elastic and soft as compared with the rigid and hard shells of untreated eggs.

Injecting the eggs or larvae into the duodenum of the chick involved anesthesia and operative surgery. In Experiments I and II (Table 1), 0.0030 grains of sodium pentobarbital was given intraperitoneally. In some groups as many as 50% of the chicks were killed although the usual mortality rate was about 10%. Chloral hydrate given *per os* according to the method of Lee (1953) also was unsatisfactory but ether, used in Experiment III, proved to be a satisfactory anesthetic.

Injections of eggs or larvae into the duodenum were made during laparotomy without aseptic conditions. This technique consisted of picking up with a forceps the loop of the duodenum proximal to the gizzard and pushing a 10-gauge hypodermic needle into the lumen of the duodenum. A tuberculin syringe was then inserted into the needle and the measured dose of eggs or larvae was pipetted into the syringe. The insertion of the syringe plunger forced the contents of the syringe into the lumen of the duodenum. The plunger was then removed and any remaining eggs or larvae were washed from the sides of the syringe by tap water and forced into the duodenum.

The criterion used to measure the effect of the *A. galli* egg shell on the survival of larvae in the host was the number of worms recovered at the end of the experiments. A statistical sample based on surviving chickens was used in all of the

TABLE 1.—*Average number of* A. galli *recovered from chicks following various methods of infection*

Experiment No.	Methods of Infection and Treatment of Eggs					
	Orally			Duodenally		
	Untreated (CO)	De-shelled (TO)	Hatched (HO)	Untreated (CD)	De-shelled (TD)	Hatched (HD)
I	1.8	0.4	0.4	4.0	3.2	2.6
II	9.7	2.5	0.6	8.3	5.2	7.5
III	7.7	2.9	0.8	7.2	8.8	5.0
Average	6.4	1.9	0.6	6.5	5.7	5.0
Total number surviving chicks	45	49	40	34	40	35

experiments (Table 1). All data on worm numbers were examined by the analysis of variance and the 5% probability level was considered significant.

RESULTS AND DISCUSSION

The data recorded in Table 1 reveal several important differences between the numbers of recovered worms. There was no significant difference between the average number of worms recovered from Groups TO and HO. However, there were significantly fewer worms in these two groups than in the other four groups; this is evidence that the intact shell protects the larva as it passes through the esophagus, crop, proventriculus, and gizzard. The lack of statistically significant differences between the numbers of worms recovered from Groups CO, CD, TD, and HD indicates that the sodium hypochlorite-sodium hydroxide solution had no detrimental effect on the larvae. A significant difference existed between the results of Experiment I and those of Experiments II and III. This difference may be due to the fact that too few chicks survived in Experiment I to give a sample comparable to Experiments II and III.

No data are available to show in which part or parts of the digestive tract, anterior to the duodenum, the hatched larvae or the larvae within the treated eggs were destroyed. Since the consensus is that little or no enzymatic digestion takes place in the chicken digestive tract above the duodenum (Dukes, 1947 and Sturkie, 1954), the larvae in the de-shelled eggs or the free larva probably were destroyed by the activity of the gizzard with its keratinized epithelium. The chickens received no grit. Grit could conceivably enhance the destructive action of the gizzard on the larvae.

An average of only 0.6 worms in a total of 40 chicks fed larvae orally (Group HO) showed a low survival rate for these larvae. Possibly, the few worms that were found developed from unhatched larvae, which may have been present in the inocula. In agreement with Guberlet (1924) and Ackert (1931), it can be concluded that chickens rarely become infected with *A. galli* by ingesting hatched larvae.

There was no significant difference between the number of worms in Groups CO and CD, indicating that the hatching of *A. galli* larvae in the small intestine does not depend upon any action by the organs anterior to the duodenum. Frenzen (1954), using *in vitro* tests, reported that proteolytic digestion of the egg shell of this worm in the presence of chicken gall, mechanical pressures, and osmotic phenomena can promote hatching of larvae. It seems likely from the present study that the 2- and 3-day period for maximum hatching of eggs *in vitro* (Frenzen, 1954) far exceeds the time necessary for hatching *in vivo* for the following reasons. A range of 4 to 26 hours has been reported for the passage of ingesta through the digestive tract of chickens. There is reason to believe that the rate of food passage in the young chicks was approximately 4 hours (Kaupp and Ivey, 1923). Furthermore, the time for egg passage could have been reduced because they were injected into the duodenum of chickens of Groups CD and TD. Possibly, the mechanical effect of peristalsis and the increase in larval activity as the temperature within the egg reaches that of the host may account for more rapid *in vivo* hatching.

Studies are now being initiated to investigate possible climatic and/or edaphic factors which may affect the egg shell of *A. galli* and the infectivity of unhatched larvae.

SUMMARY

Hatched larvae of *Ascaridia galli* or larvae surrounded by the vitelline membrane of de-shelled eggs, when given orally to chickens, have a much lower survival rate than comparable larvae or eggs injected directly into the duodenum.

Normal eggs, which have not passed through the esophagus, crop, proventriculus, and gizzard, can hatch when placed directly into the duodenum of the chicken.

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THE ULTRASTRUCTURE OF *LEISHMANIA DONOVANI*

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The structure of the intracellular form of *Leishmania donovani* as seen by the light microscope consists of a nucleus and usually a rod-like kinetoplast (Wenyon, 1926) within a homogeneous mass of cytoplasm, while the extracellular or culture form has, in addition to these structures, an anterior flagellum. The intracellular form is usually round or ovoid measuring 2–4 microns; the extracellular form measures about 14–20 microns in length and from 1.5–3.5 microns in breadth. Because of the small dimensions of the protozoan parasite and the limited resolution of the light microscope, relatively little information is available on its fundamental organization. With the great resolving power of the electron microscope, several workers have attempted to study its ultrastructure with this instrument. The studies of Emmel, Jakob and Golz (1942), Sen Gupta *et al.* (1951), Das Gupta *et al.* (1954) of this species, and Lofgren (1950) of *Leishmania tropica* using electron microscopy of the culture forms have failed to yield new information on the fine internal morphology because the parasites were examined as whole specimens. The thickness of the specimens does not allow for adequate electron beam penetration, and the parasites appear as dense masses. In this study however, this problem is eliminated by the use of thin sectioning. Fine structural detail is observed in these preparations in both the extra- and intracellular forms. The accumulation of structural knowledge may enable us to understand the relationship of host cells and leishmania; i.e., the biology of this intracellular parasitism.

This paper presents only a few observations of this host-parasite relationship and further work is necessary to make a thorough study of this problem.

MATERIALS AND METHODS

Intracellular parasites were obtained from infected hamster spleen and the extracellular forms from NIH diphasic medium cultures. The culture forms were washed in Geys' salt solution (Gey and Gey, 1936) before fixation. The culture forms and infected spleen material were fixed in 1% veronal-buffered osmium tetraoxide, pH 7.2–7.4 (Palade, 1952), dehydrated by a graded series of ethyl alcohol and embedded in a partially polymerized mixture of one part of methyl to four parts of butyl methacrylate. The embeddings were incubated in the oven overnight at 45 degrees centigrade, sectioned with glass knives on a Servall microtome, mounted on screen and examined in a RCA, Model EMU electron microscope.

OBSERVATIONS

Intracellular Form

The three leishman bodies shown in Figs. 2, 3 and 4 consist of three serial sections of the same three parasites within the cytoplasm of the host cell in hamster spleen. These three parasites are lying in close proximity to the nucleus of the host cell and will hereafter be referred to as *Leishmania A, B* and *C*.

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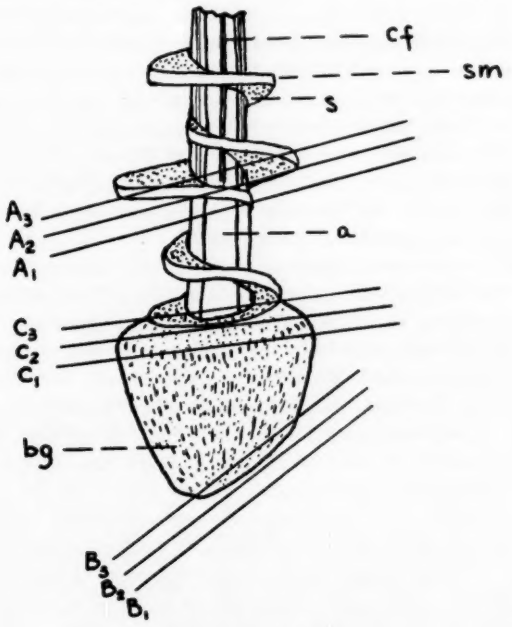
Leishmania A. Fig. 2 shows a leishman body with a cell membrane which is distinct from the cytoplasm of the host cell. At this level of sectioning, the leishman body reveals the following structures: 1) A nucleus with fine, granular nucleoplasm surrounded by a nuclear membrane. The nucleoplasm is a homogeneous mass with a clear vacuole on one side. No evidence of a nucleolus or karyosome is recognized in this body. 2) A dense ring with a hollow center whose structure and relationship will be clearly revealed in Figs. 3 and 4 as the axoneme of the reduced flagellum. 3) Three dense, structureless droplets, unequal in size, are tentatively regarded as fat bodies. Droplets similar to these have been described in *Trypanosoma cruzi*



EXPLANATION OF FIGURES

Fig. 1 Diagrammatic interpretation of the proximal flagellum and basal granule structures of the leishman body. The structures observed in serial sections of Figs. 2, 3 and 4 are interpreted with reference to Fig. 1 in the following manner:

Leishmania A. Fig. 2 represents a cross section at the level which corresponds to the junction of two adjacent coils of the sheath. The amount of protoplasm enclosed by the sheath membrane is therefore reduced to a minimum making it barely visible. Enclosed within the sheath is the dense axoneme, which appears as a dense ring in cross section. Figs. 3 and 4 are serial sections distal to the basal granule. Fig. 3 shows a cross section of the axoneme and one coil of the sheath. Fig. 4 shows the cross section of the axoneme and two opposite coils of the sheath, one on each side of the axoneme which appears as one continuous circular tube.

Leishmania B. Figs. 2 and 3 are sections below the level of the basal granule. In Fig. 4, a small portion of the basal granule is sectioned giving the semi-circular appearance.

Leishmania C. Figs. 2 and 3 represent cross sections of the basal granule in the transitional region from which the extended flagellum arises. Fig. 4 represents the first cross section of the flagellum immediate to the basal granule.

The three leishman bodies represent three different levels of sectioning through the cell. The presence and absence of certain structures in certain cells would be very difficult to interpret without the aid of serial sections.

by Meyer and Porter (1954), and they found that the content of these bodies could be removed by xylene. 4) Vacuoles of various sizes are visible in the fine granular cytoplasm. These vacuoles are distributed at random throughout the cell.

In Fig. 3, a follow-up on the continuity of the structures described in Fig. 2 reveals the following changes: 1) The nucleus shows up more clearly with a double membrane enclosing the nucleoplasm. The nucleoplasm is finely granular and "patchy," and lying in an eccentric position is an aggregate of densely packed, rather homogeneous granules which is considered to be the nucleolus or karyosome. The structure of nucleoli has been studied by Borysko and Bang (1951). They found a wide variety of forms; included was a homogeneous nucleolus of an endothelial cell which resembled that observed in *Leishmania donovani*. 2) The dense ring with the hollow center (axoneme) is still present; however, in addition to this structure, there is another less dense granular ring partially surrounding the axoneme. This is analogous to the sheath of several protozoan flagella described by Brown (1945) and of *Trypanosoma cruzi* by Meyer and Porter (1954). 3) The three dense droplets are still present and they are lying in the same relative positions within the leishman body. 4) Vacuoles of various size are still seen and are distributed at random throughout the cytoplasm.

Fig. 4 shows a very interesting development of two important structures of the organism. 1) The nucleus has a nucleolus within it. The nucleoplasm is finely granular and aggregates of nucleoplasm originating from the central nucleolus form a linear, ray-like pattern within the nucleus. 2) The dense ring structure (axoneme) now has a central body within it. This central body is very similar to the internal filaments of several cilia types studied by Fawcett and Porter (1954). The sheath with its well defined membrane encircling the entire axoneme is a coiled structure. The transition of the varying size and thickness of the sheath may be clarified in the diagrammatic interpretation shown in Fig. 1. 3) Only two of the fat droplets are present. 4) The cytoplasmic vacuoles appear as described above.

Leishmania B. In Fig. 2 this cell consists of a nucleus without a nucleolus and four fat droplets. The cytoplasm is a homogeneous mass of fine granules enclosed in a double membrane. The outer membrane seems to be a smooth layer, but the inner layer seems to be composed of evenly-spaced fine ridges. Fig. 5 shows this structure very clearly. Fig. 3 shows a similar picture of all structures as described in Fig. 2. Fig. 4 shows changes and addition of structures described in Figs. 2 and 3. The nucleus now has an eccentrically located nucleolus, which appears as a dense homogeneous structure composed of fine granules. There is a semicircular ring with less dense cytoplasm distinct from the cell cytoplasm. There is the appearance of a structure not observed in the cells described above. A reconstruction of the flagellum structure, Fig. 1, and an electron micrograph of a longitudinal section of the extended flagellum of a leptomonad form indicates the relationship of such a structure. This structure is a section of the base of the basal granule. Fat bodies and vacuoles are present in the cell cytoplasm. The double membrane with a ridged inner surface is still evident here.

Leishmania C. In Fig. 2, two prominent ovoid structures almost equal in size are clearly seen in this cell. Although both these structures are composed of fine granular substance, they are distinctly different from each other. One of them is

easily recognized as the nucleus and the other is identified as the basal granule from which the flagellum arises. Each has its own distinct membrane. The nucleus shows a denser nucleoplasm at the periphery of the nuclear membrane than in the central area and there is no evidence of a nucleolus at this level. One dense fat body and several small vacuoles are scattered throughout the cytoplasm. The cell membrane is a double one.

In Fig. 3, the dimensions of the cell nucleus and basal granule are increased. The nucleoplasm is denser and the fine granules are evenly distributed throughout, but there is still no evidence of a nucleolus. The granules in the basal granule are very sharp and distinct; they are centrally located and spread out as a band on the long axis of the basal granule.

In Fig. 4, the structures in the cell show a marked change from the previous series. The nucleus has a fairly homogeneous nucleoplasm and a faint indication that a very small portion of the nucleolus is present. The basal granule is no longer seen; in its place the dense ring of the axoneme is completely surrounded by a sheath. The flagellar structure has arisen from the basal granule. Again, reference to Fig. 1 will clarify the reconstruction of the basal granule-flagellum relationship. Vacuoles of various sizes are seen throughout the cytoplasm. The single fat droplet in Fig. 2 and 3 is no longer visible; instead, one new droplet may be located near the nucleus. The double membrane which surrounds the cell seems to be separated at one end of the cell: the inner layer still surrounds the usual dense cytoplasm of the cell; the outer layer seems to extend at one end of the cell and encloses a relatively clear cytoplasm. This phenomenon has been observed among other leishman bodies; Fig. 6 illustrates another such observation. The significance of this clear area is not apparent. Further study is necessary to clarify these random observations.

Extracellular Form

Fig. 7 shows two leptomonad forms cut at different angles. The cell above shows a rounded basal granule which becomes constricted at one end to give rise to the flagellum. The flagellum consists of a cylindrical tube-like axoneme which extends beyond the cell membrane as a free flagellum. A central filament within the axoneme begins at a short distance from the basal granule and continues along with the axoneme. Surrounding the axoneme is a loosely coiled sheath which begins at the base of the axoneme and continues throughout its entire length. The cytoplasm is granular and there is no evidence of fat bodies or vacuoles. The nucleus is not visible because of the level of the section.

The cell below is a longitudinal section. The cytoplasm is granular and homogeneous throughout the cell. Within this cell are the nucleus, the basal granule, axoneme and sheath. The nucleus has a nuclear membrane and within it is a dense, homogeneous nucleolus. The basal granule is a rounded body, and within it is a band of uniform short filaments at right angles to the axoneme. The significance of this band is obscure. The axoneme and sheath are present but only to the edge of the cell membrane; the free end of the flagellum has been deleted at this level of sectioning.

The evidence of "cell wall" with fine, longitudinal striations in *Leishmania tropica* described by Lofgren (1954), the sub-pellicle fibers or striations over the body surface of *Trypanosoma cruzi* described by Meyer and Porter (1954) and

the myoneme fibrils of *Leishmania donovani* noted by Das Gupta *et al.* (1954) are not clearly indicated in this electron micrograph, probably because the techniques employed by these workers were different from those employed here.

DISCUSSION

The technique of thin sectioning of *Leishmania donovani* for electron microscopy has revealed some details of morphology not obtainable by the whole-mount techniques employed by previous workers. No drastic treatment of specimens by acid hydrolysis (Das Gupta *et al.*, 1954) or by osmotic rupture by distilled water (Lofgren, 1950) were used in this study. Thus, artefacts have been minimized.

Since the structures of the leishman body and the leptomonad form are similar, the morphology of these structures may be compared.

In the Protozoa, the limiting layer which gives the organism its shape has been variously called the pellicle or periplast. It may be smooth or ridged. In the studies of several flagellates by electron microscopy, Saxe (1947) described the "spiral striations of the pellicle" in *Euglena gracilis*. Lofgren (1950) described "the cell wall of the *Leishmania* cells possessed fine, longitudinal striations." Kleinschmidt and Kinder (1950) found fibrillar structure of the periplast in *Trypanosoma lewsi* and *Trypanosoma brucei*. Kraneveld, Houwink and Keidal (1951) found that the periplast contains longitudinal and nearly parallel fibrils in *Trypanosoma evansi*. Wolken and Palade (1953) reported a ridged pellicle of *Euglena gracilis*. Meyer and Porter (1954) observed a body membrane with fibrils in the cytoplasm just beneath the membrane in *Trypanosoma cruzi*. Das Gupta *et al.* (1954) described "longitudinal myoneme fibrils arranged in interconnecting bundles in the periplast" of *Leishmania donovani*. These observations on the fibrillar nature of the cell membrane in a variety of flagellates indicate a close similarity of the cell membrane structure among them, whether they be free-living or parasitic. Most of thse observations were made on whole specimens, and in the observation of sectioned material by Wolken and Palade (1953), the ridged pellicle of *Euglena* was like a "scalloped" edge on the outside of the cell membrane. The observations of the intracellular phase of *Leishmania donovani* in the present study reveal a double cell membrane, with a smooth layer on the outside and a finely ridged surface in the inner layer. This conforms with the description given by Meyer and Porter (1954) of *Trypanosoma cruzi*.

The nuclei of Protozoa, like other types of cell nuclei, are bounded by distinct nuclear membranes. The workers using whole mount technique have provided little or no information on the structure of the nucleus because the organisms are usually too dense for adequate electron beam penetration. By using the HCl hydrolysis technique on *Leishmania donovani*, Das Gupta *et al.* (1954) obtained electron micrographs showing an oval body that is less dense than the rest of the cytoplasm. A small dark body within the nucleus is presumed to be the karyosome. Sectioned material provides a much clearer picture of this structure. The study of *Toxoplasma* by Gustafson *et al.* (1954) shows the nuclei in a variety of shapes, but all show a consistent dense mass within the nucleus. The present study of *Leishmania donovani* confirms the presence of the nuclear membrane; and in optimum sections, a double nuclear membrane may be seen. Within the resting nucleus, a karyosome may be seen. The absence of the karyosome in some cells is most probably due to the level of sectioning.

Many studies have been made on the flagellar structure in parasitic and free-living flagellates. All reveal the same basic structure, consisting of an internal axoneme and a sheath surrounding it. Brown (1945), in study of various flagella, found that the axoneme was composed of fibrils. He was uncertain as to the number which constitute an axoneme. Saxe (1947) also reported a number of longitudinal components in the axoneme of *Euglena gracilis*. Lofgren (1950) in a study of *Leishmania tropica*, Kleinschmidt and Kinder (1950) in studies of *Trypanosoma lewsi* and *Trypanosoma brucei*, and Kleinschmidt and Schleich (1951) in studies of *Trypanosoma brucei*, reported that the axoneme was composed of fibrillae. No mention was made of the number which make up the axoneme. There are other workers who give numerical counts on the fibrils constituting the axoneme. The numbers vary because each study was of a different flagellate. Whether this is a true difference between species is difficult to evaluate at this time. Until improvements in techniques allow good, clearcut electron micrographs, we cannot establish whether the fibrils which constitute an axoneme have a basic number or vary with the organism. Examples of this controversy will be presented as follows: Kraneveld *et al.* (1951) reported nine parallel isodiametric fibrils in the axoneme of *Trypanosoma evansi*, Wolken and Palade (1953) found eleven elementary filaments in *Euglena* and *Poteriochromonas* in their axonemata. Meyer and Porter (1954) found five or nine fibrils in the axoneme of *Trypanosoma cruzi*. Das Gupta *et al.* (1954) found a maximum of nine fibrils in *Leishmania donovani*. In the present study of *Leishmania donovani*, the fibrillar structure is confirmed; however, the exact number is not readily counted. The fact remains that the axoneme is made up of fibrils.

Besides the fibrillar axoneme of flagellates, studies of Schmitt, Hall and Jakus (1943) and Schmitt (1944) show that sperm tails and cilia have similar ultrastructure. Their observations showed the presence of subfibrils regarded as the axoneme, which were in turn surrounded by a sheath. The sheath bounded the subfibrils in a tightly coiled helix. Similar regular banded structure was found in the shaft portion of *Paramecium caudatum*. More recently, Fawcett and Porter (1954) confirmed the same basic structures in the cilia of epithelial cells from mollusc, amphibia, mouse and man.

The sheath always surrounded the axoneme in all the studies mentioned above of flagellates. A helix was the common description of its structure. Some workers just mention a membranous sheath surrounding the axoneme. This study of *Leishmania donovani* shows a loosely coiled sheath, (Fig. 7). This would appear to be similar to the coiled fiber sheath described by Brown (1945); however, a reconstruction of the sheath structure from the serial cross sections proves this is not so of *Leishmania donovani*. Instead, it is more like a coiled band of protoplasm bounded by a thin membrane. The diagrammatic reconstruction (Fig. 1) from the serial sections shows this interpretation.

Not to be dissociated from the flagellum proper, is the basal granule. This structure has been referred to by other names such as the basal body, basal corpuscle, blepharoplast, kinetonucleus, kinetoplast and others. Early workers observed a dense body which was always more or less closely associated with the flagellum. Some observers have reported that this structure has often been associated with another granule and may be regarded as a parabasal body. Chemical studies

conducted on flagellates have shown Feulgen-positive material in these granules; this has led workers to regard this as a second nuclear structure.

At the present time, very little information is available on the structure of the basal granule as revealed by electron microscopy. The difficulty lies in the limited techniques which have been used to reveal the internal structure of flagellates. The thin sectioning technique for electron microscopy has been very useful in revealing structures not encountered before, but, at the same time, new problems of interpretation and identification are created. Spherical bodies in cells showing no other structural detail than a nucleus have been labelled as the blepharoplast (Meyer and Porter, 1954) or the blepharoplast and parabasal bodies (Das Gupta *et al.*, 1954). These descriptions show no relationship to other structures in the cell. Kleinschmidt and Schleich (1951) state that the flagellum does not take its origin from the blepharoplast or the basal granule. Wolken and Palade (1953), studying sectioned material of *Poteriochromonas,* show the basal body at the base of the flagellum.

A cross banding was observed in the basal granule of the flagellate form in this study. What its relationship is to the basal granule or flagellum is not clear. Descriptions of structures resembling this cross banding have been observed in other flagellates. Kraneveld *et al.* (1951) found a "collar like" structure at the base of the flagellum of *Trypanosoma evansi* and they think that this structure is in all probability identical to the "ring-like" structure at the base of the axoneme of *Leishmania tropica* described by Lofgren (1950).

Besides the basal granule of the flagellates, there is a similar structure at the base of the cilium. Fawcett and Porter (1954) in studies of various ciliated epithelia, found two different basic types among them: (1) A separate basal corpuscle and cilium structure held together by cell or ciliary membranes (found in the mollusc and amphibia). (2) A continuous basal corpuscle and cilium structure (found in mammals). The basal granule-flagellum component in *Leishmania donovani* observed in this study is similar to the type found in mammals. The cross banding observed in the flagellates may be similar to the periodic cross banding in the basal corpuscle at the cilium in *Anodonta* described by Worley, Fischbein and Shapiro (1953). The validity of this structure is denied by Fawcett and Porter (1954). This does not seem to be an artefact since Lofgren (1950) saw this by phase microscopy. As yet, there is no proof that these are the same structures and further work would be required to settle this controversy.

The cytoplasmic inclusions found in *Leishmania donovani* are those commonly found in other types of cells; namely, fine granules, vacuoles of various sizes, osmophilic droplets and mitochondria (?). The components of the osmophilic droplets have not been definitely established. Meyer and Porter (1954) found that the contents of the osmophilic droplets of *Trypanosoma cruzi* could be dissolved by xylene. This would suggest the lipoidal nature of these droplets. In this study of *Leishmania donovani,* the number and size of these droplets varies from cell to cell. They are not found in some sections. This may be due to the level of sectioning, or, the cell does not have any. As yet, experiments have not been carried out to determine whether these droplets are really fat. However, Read and Chang (1955) made observations by light microscopy on tissue cultures on chicken macrophages infected with *Leishmania donovani* from culture. Using Sudan III

to demonstate fats in infected and uninfected macrophages, they found that the infected macrophages possess smaller fat droplets and the staining intensity was reduced when compared with the uninfected macrophages. What this means in terms of host-parasite relationship would invite further study.

Small bodies in the cytoplasm may be mitochondria, but their internal structure is not clear enough to label them as such.

SUMMARY

1. The ultrastructure of the intra- and extracellular phases of *Leishmania donovani* have been studied by the use of thin sectioning and electron microscopy. Fine structural details revealed here have not been observed by previous workers using the whole-mount technique.

2. The intracellular phase or the leishman body found within the hamster spleen cell possesses the following structures:

a) A limiting, double cell membrane which is distinct from the cytoplasm of the host cell: A smooth outer layer and a ridged inner layer.

b) A smooth double nuclear membrane enclosing the finely granular nucleoplasm. In some sections, a homogeneous nucleolus or karyosome may be seen within the nucleus.

c) A reduced flagellum consisting of a fibrillar axoneme surrounded by a coiled band (not coiled rope as described by others) of protoplasm of the flagellar sheath.

d) A rounded basal granule located at the proximal end of the flagellum.

e) Cytoplasmic inclusions similar to those found in other types of cells: Fine granules, vacuoles of various sizes, osmophilic droplets and mitochondria (?). The components of the osmophilic droplets have not been established.

3. The extracellular phase or the leptomonad form shows slight modification of some of the structures described for the intracellular phase, as follows:

a) The cell membrane is a smooth layer without an inner ridged layer. The longitudinal striations on the body surface observed by other workers in whole-mount specimens (not observed in this phase) have been discussed.

b) The flagellum extends beyond the cell membrane as a free flagellum. The fibrillar axoneme is evident; a loosely coiled band (the protoplasmic sheath) surrounding it begins at the base of the axoneme and continues throughout its entire length.

c) The basal granule has within it a cross banding whose significance is obscure.

d) Osmophilic droplets were not seen in the specimens studied. Whether this is a true absence of them in this phase of their life cycle is difficult to evaluate at this time.

4. It is concluded from these observations that the intra- and extracellular phases of the parasite possess the same basic structures.

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EXPLANATION OF PLATES

PLATE I

Figures 2, 3 and 4 show three serial sections of three leishman bodies labelled A, B and C. They are lying within the cytoplasm of a hamster spleen cell. Axoneme (a), basal granule (bg), central filament (cf), host cell nucleus (hcn), lipid droplet (ld), nucleus (n), karyosome or nucleolus (nu), and sheath (s). Magnification: 19,700×.

PLATE II

Fig. 5. A leishman body showing a distinct double cell membrane: a smooth outer cell membrane (osm) and an inner ridged cell membrane (irm). A nucleus (n) showing distinct nuclear membrane (nm). Within the nucleus is a dense homogeneous karyosome or nucleolus (nu). Magnification: 35,400×.

Fig. 6. Shows a leishman body with an extended outer cell membrane enclosing a relatively clear cytoplasm. The nucleus (n) is present and two lipid droplets (ld). Magnification: 19,700×.

Fig. 7. Shows two leptomonad forms cut longitudinally. The one above shows an extended flagellum. Axoneme (a), basal granule (bg), cross band (cb), nucleus (n), karyosome or nucleolus (nu) and sheath (s). Magnification: 19,700×.

PLATE I

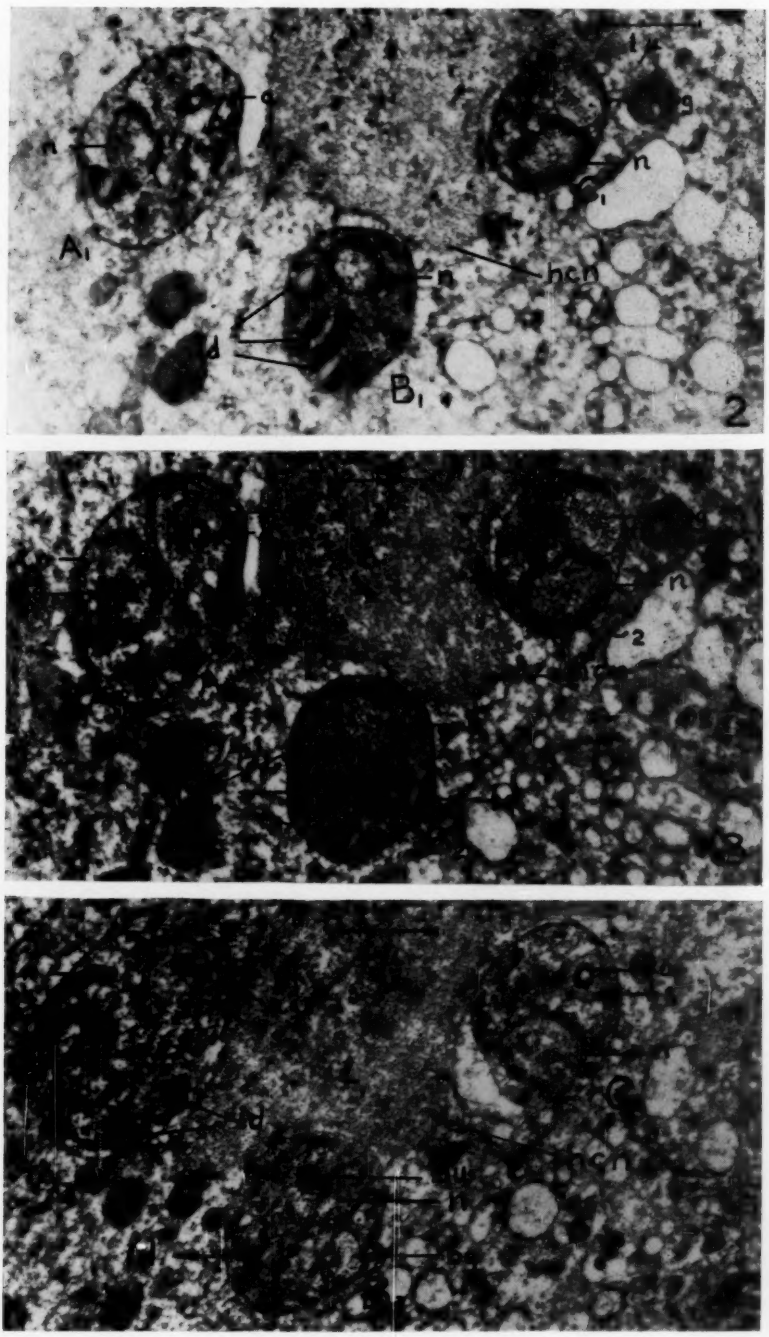
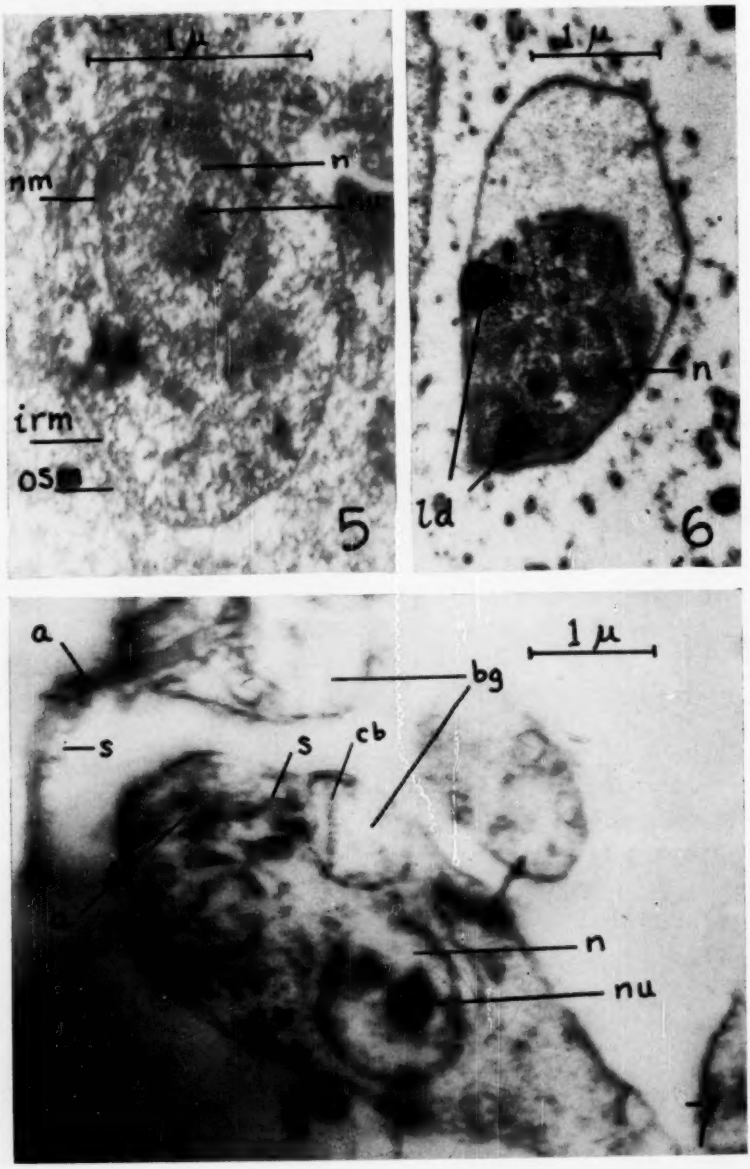


PLATE II



OBSERVATIONS ON VECTORS OF SCHISTOSOMIASIS MANSONI KEPT OUT OF WATER IN THE LABORATORY. I.*

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Recently, the habits and seasonal cycles of *Australorbis glabratus* and *Tropicorbis centimetralis,* intermediate hosts of *Schistosoma mansoni,* were studied intensively in the field in Brazil (Olivier and Barbosa, 1955a, b). In the course of these studies, the observations of Barbosa and Dobbin (1952) concerning the ability of *A. glabratus* to live out of water were confirmed and extended, and similar observations were made on *T. centimetralis.* In conjunction with these field studies, the snails were also studied in the laboratory where the environmental conditions were more constant and under a measure of control and where individual snails could be observed for long periods of time. Some of the experiments were designed to determine how long the two vector species could live out of water in the laboratory and whether there are strain differences within the species with respect to their ability to do this. In one group of such experiments, the snails were maintained without water in unglazed clay jars for long periods of time. The results of these experiments will be described here.

MATERIALS AND METHODS

Northeastern Brazil has a marked seasonal rainfall cycle (Olivier and Barbosa, 1955a), and as a consequence, in addition to the permanent bodies of water, there are many temporary streams and pools which contain water only in the wet season which lasts from March or April to July or August. The schistosome vectors inhabit both temporary and permanent bodies of water; snails for the laboratory experiments were drawn from both types of habitats.

The snails, whether collected from wet or from dry habitats, were always brought into the laboratory in metal cans without water. Snails collected from the water were tested for infection by isolating them in water one day after which they were held out of water in moist clay jars until used. Snails collected from dry habitats could not be tested for infection without wetting them; so they were left untested and were maintained in moist clay jars until used. All snails came from populations known to have a low prevalence of infection with *S. mansoni* or other trematodes. The snails were always used within a few days after collection and no snail known to be infected was used.

In all experiments, except Experiment 18, the snails were kept in unglazed clay jars about 15 cm. in diameter and about 7 cm. deep (Fig. 1). The jars were always covered with loose fitting gauze lids, and in almost all cases, the jars con-

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could be maintained at a relatively constant level and measured with a hygrometer.

In all experiments, the snails were examined periodically for viability by passing a strong beam of light through the shell in a darkened room. This was considered the best method for judging the viability of the snails without disturbing them or putting them in water. The accuracy of the method was tested by putting snails in water after they were examined in the light beam. The method proved to be reasonably accurate, but occasionally a dead snail was judged to be alive. In a living snail retracted into the shell, the part of the shell occupied by the snail body is opaque and the free boundary of the foot makes a sharp, straight line readily seen through the shell in transmitted light. In dead snails the free margin of the foot is not straight and sharp, and the part occupied by the snail body is not uniformly opaque because of shrinkage of the tissues away from the shell or the formation of gas and fluid within the body after death. As the snails dried they re-

FIG. 1. Experiment 11, in October, 1953, showing the clay jar and the pan in which it was kept. A small amount of water in the pan kept the bottom of the jar wet at all times. The gauze cover is not shown.

tracted farther and farther into the shell, and it became increasingly difficult to tained nothing but the snails. In Experiments 9 and 18, the snails were maintained on sand.

All experiments were conducted at laboratory temperature which varied little. During the dry season the temperature ranged from 27 to 30° C., while during the remainder of the year the range was from 25 to 28° C. The relative humidity of the laboratory was more variable, but it usually ranged from 65 to 75 percent during the dry season and from 75 to 90 percent in the wet season.

In certain experiments, the jars were kept on the laboratory bench top, and the temperature and humidity conditions for these experiments were approximately those of the laboratory. In other experiments, the jars were set in pans of water which raised the relative humidity within the jars to 90–95 percent. In still other experiments, the jars were placed in large wooden cabinets where the humidity

TABLE I—*Survival out of water of* A. glabratus *and* T. centimetralis *collected from water in natural habitats*

Experiment	Starting date	Snail species	Source of snails	Nature of habitat	Relative humidity during experiment (%)	Number of snails	Remarks
1	Dec. 19, 1953	*T.c.*	Iputinga, Recife	Temporary pools	90-95	79	67 survived 48 days 9 survived 99 days 0 survived 113 days
2	Feb. 4, 1954	*T.c.*	Lagoa Caiçara near Vitória	Permanent lake	75-80	400	Heavy losses in first 3 weeks 3 survived 99 days (water test)
					90-95	400	Heavy losses in first 3 weeks 1 survived 99 days (water test)
3	Feb. 5, 1954	*T.c.*	Casa Forte, Recife	Concrete lily pool	75-80	291	All dead in 2 days
					90-95	291	All dead in 2 days
4	Feb. 6, 1954	*T.c.*	Apipucos, Recife	Permanent lake	70-80	240	None lived 9 days
5	Feb. 8, 1954	*T.c.*	Vitória de Santo Antão	River	75-80	367	Heavy losses in first 2 weeks 18 survived 109 days (water test)
					90-95	367	Heavy losses in first 2 weeks 2 survived 109 days (water test)
6	Feb. 22, 1954	*T.c.*	São Lourenço	River	75-80	310	Heavy losses in first week 4 survived 43 days (water test)
					90-95	400	Heavy losses in first week 34 survived 43 days (water test)
7	July 9, 1953	*A.g.*	Arruda, Recife	Field temporarily flooded	90-95	60	11 survived 96 days 2 survived 138 days
8	June 1, 1953	*A.g.*	Bairo Novo, Recife	Permanent pool; depth variable	70-80	63	33 survived 57 days 3 survived 113 days
9	July 24, 1953	*A.g.*	Arruda, Recife	Field temporarily flooded	90-95	213	141 survived 113 days 15 survived 152 days 1 survived 193 days
10	Dec. 26, 1953	*A.g.*	Bairo Novo, Recife	Permanent pool; depth variable	70-80	132	118 survived 20 days 64 survived 41 days 13 survived 83 days 1 survived 104 days
11	Feb. 5, 1954	*A.g.*	Bairo Novo, Recife	Permanent pool; depth variable	85-90	200	185 survived 20 days 68 survived 63 days 14 survived 105 days (water test)
					75-80	200	194 survived 20 days 24 survived 63 days 4 survived 105 days (water test)

recognize dead snails. Therefore, in some of the experiments, groups of snails were tested in water and then destroyed while, in other instances, all survivors were tested in water at the end of the experiment (the water test).

EXPERIMENTS WITH SNAILS COLLECTED FROM THE WATER OF NATURAL HABITATS

Eleven experiments were conducted with *A. glabratus* and *T. centimetralis* collected from the water. Some were taken from permanent pools or streams and others were collected during the rainy season from temporary bodies of water. *T. centimetralis* ranged from 2 to 10 mm. in diameter and the *A. glabratus* from 10 to 24 mm. Results of the experiments were presented in Table I.

EXPERIMENTS WITH SNAILS COLLECTED FROM DRY NATURAL HABITATS

Snails collected from dry natural habitats were studied in 6 experiments. In each case the snails were from protected locations in vegetation and debris at the ground level. The *T. centimetralis* came from tussocks of *Cyperus ligularis* L. in a field in Salgadinho, Recife (see Olivier and Barbosa 1955b). They were relatively dry when collected and were usually retracted into the shell about one-half of the terminal whorl. In both 1953 and 1954, the field dried in September and remained dry until the beginning of the following rainy season which began in April. The *A. glabratus* were from a meadow near Paulista, Pernambuco (see Olivier and Barbosa 1955a). They were collected from a dense mat of moist vegetation and plant debris. Coming from such a humid environment, they were not severely dehydrated, and most of the snails were retracted only about one quarter of the terminal whorl. Some had, in the unoccupied portion of the shell, very thin and extremely fragile membranes. These were always incomplete, but some had more than one. Their efficacy in protecting the snails against water loss is unknown. The habitat from which the *A. glabratus* came died in November, 1954 and remained without standing water until April, 1954. All the snails used in these experiments may be considered to be the survivors of much larger populations present before the habitats dried. The *T. centimetralis* were all 5 to 8 mm. in diameter, and the *A. glabratus* were 25 to 31 mm.

The results of these experiments are presented in Table II.

COMPARISON BETWEEN TWO STRAINS OF *A. glabratus* KEPT OUT OF WATER UNDER IDENTICAL CONDITIONS

In a further experiment designed to determine whether there are strain differences with respect to the ability of the snails to survive out of water, two strains of *A. glabratus* were kept out of water under identical conditions, and their survival patterns were compared. Three experiments were conducted using snails from Paulista, Pernambuco and from Salvador, Bahia. The snails from Paulista were taken from a temporary pool. The snails from this locality were known to be able to live out of water for long periods under certain conditions (see Experiments 15, 16, and 17; Barbosa and Dobbin, 1952; and Olivier and Barbosa (1955a, 1955b). The snails from Salvador were collected from the Dique de Tororó, a lake within the limits of the city. This body of water never dries up, and its level does not

TABLE II—*Survival out of water of* A. glabratus *and* T. centimetralis *collected from dry natural habitats*

Experiment	Starting date	Snail species	Length of time habitat had been without standing water (months)	Relative humidity during experiment (%)	Number of snails	Remarks
12	Mar. 28, 1953	*T.c.*	6.5	65-90	37	13 survived 73 days 10 survived 101 days 3 alive at 228 days (water test) 1 alive at 321 days (water test) 1 alive at 385 days (water test) 1 alive at 419 days (water test)
13a	Dec. 2, 1953	*T.c.*	2.5	90-95	170	150 survived 177 days (water test)
13b	Dec. 15, 1953	*T.c.*	3.0	90-95	52	40 survived 46 days
14	Feb. 14, 1954	*T.c.*	5.0	90-95	321	43 of 50 survived 90 days (water test) 151 survived 146 days (water test)
15	Dec. 23, 1953	*A.g.*	1.0	90-95	153	139 survived 100 days 79 survived 207 days
16	Jan. 4, 1954	*A.g.*	1.5	70-80	17	13 survived 108 days (water test) 5 survived 196 days (water test)
17	Feb. 10, 1954	*A.g.*	2.5	85-90	63	59 survived 98 days 51 survived 158 days (water test)

fluctuate to any great extent. The capacity of these snails to survive out of water was not known when the experiments were begun. On the day of collection, the Salvador snails were packed in moist leaves and carried to Recife by air courier in the plane cabin. On arrival in Recife they were placed in large holding tanks, and the experiments were begun within two days after their arrival.

Experiment 18.

In the first of these experiments, 200 snails from each locality were used. The Paulista snails ranged from 15 to 25 mm in diameter (average, 19.3) while the Salvador snails ranged from 6 to 13 mm in diameter (average 8.9). All the snails were placed on the surface of moist sand in shallow clay jars having a diameter of about 31 cm. The snails were covered with a thin layer of dry leaves and set aside on the laboratory bench top. The sand soon dried and no moisture was added. After 10 days, 100 snails from each group were tested in water. Of the Paulista snails, 89 were alive while only 56 of the Salvador snails were alive. After 36 days, the remaining snails were tested in water and 52 Paulista snails and 3 Salvador snails were alive.

Experiment 19.

In another experiment 50 snails from each locality were held at 70 to 80 percent r.h. The Paulista snails ranged from 19 to 26 mm in diameter, and the Salvador snails were about the same size and apparent age, ranging from 17 to 26 mm in diameter. The Paulista snails went 92 days without a death while half of the Salvador snails were dead in 22 days, and all were dead in 77 days. Twelve percent of the Paulista snails lived 285 days (water test).

Experiment 20.

In another experiment, almost identical with the preceding one, the snails were of approximately the same size, and the experiment differed from the other only in that there were 191 snails from Paulista and 200 from Salvador, and the humidity was 85–90 percent. The death losses were almost the same as those observed in Experiment 19. Ninety-six percent of the Paulista snails lived 131 days, 61 percent 205 days, and 4 percent 285 days. The Salvador snails died much more rapidly. Fifty-one percent lived 33 days and only 3 percent lived 77 days.

DISCUSSION

Numerous workers have observed that certain fresh-water pulmonates can survive out of water, sometimes for long periods. Some of the literature on this subject was reviewed recently in a paper dealing with the seasonal cycle of *A. glabratus* in Northeastern Brazil (Olivier and Barbosa, 1955a).

Several accounts of experiments with fresh-water snails are comparable with those that have just been described. Shadin (1926) kept two species of *Lymnaea,* three species of *Planorbis,* and *Aplexa hypnorum* out of water in the laboratory. He found that all could live out of water and that a specimen of *L. palustris* lived 79 days and a specimen of *P. planorbis* 59 days. Ross and McKay (1929) found *Lymnaea brazieri* died rapidly if it crawled out of the water. Kolpakov (1929) tested a number of pulmonates in the laboratory and found that *Planorbis corneus* could live as many as 113 days out of water, that *P. vortex* coud live 92 days, and that *P. planorbis* 91 days. Patzer (1927) and Mehl (1932) found that *Lymnaea truncatula* would live only a very short time in jars in the laboratory although it might live up to 4 to 5 months out of water under field conditions.

Archibald (1933) found that prolonged desiccation was fatal for the schistosome vectors in the Sudan, but that slow drying was better tolerated than rapid drying. Gordon, Davey, and Peaston (1934) found that *Biomphalaria pfeifferi* survived only a few days out of water in the laboratory, and they believed that snails collected from the water and suddenly subjected to drying did not live as long as those gradually acclimated to drying. Barlow (1935) concurred in this opinion. He also noted that *Bulinus truncatus* and *Biomphalaria boissyi* could live out of water in the laboratory for four and six months respectively. de Jesus and Mallari (1937) observed that *Lymnaea philippinensis* died in 16 days when kept out of water in the laboratory.

Precht (1939) found that snails kept in the laboratory on blotting paper did not survive long, but that when they were kept in a relatively moist environment, *Planorbis leucostoma* with epiphragms lived a maximum of 800 days, *P. corneus* lived as many as 285 days, and *P. planorbis* lived 368 days. On the contrary, other species lived for only a relatively short time under the same conditions.

Brumpt (1941) found that *A. glabratus* from Venezuela could live out of water in the laboratory for 50 days and concluded that this species resembles *B. boissyi* of Egypt in its capacity to live out of water. Olson (1944) kept *Stagnicola bulimoides techella* in dry open dishes in the laboratory and some lived 158 days.

The experiments described herein demonstrate clearly that the snail vectors of schistosomiasis mansoni in Northeastern Brazil may live out of water for long periods in the laboratory, but marked differences with respect to time of survival out of water were observed in the various experiments.

T. centimetralis collected from the dry vegetation in Salgadinho lived out of water for 5 to 21 months, and the same species collected from the water in other localities lived over 99 days. On the contrary, the same species, collected from other localities, lived only a few days under essentially the same conditions. Some of these differences in survival time are probably attributable to uncontrolled variables such as age and condition of the snails when collected and differences in the laboratory environment at the time of the experiments. For instance, the snails from Salgadinho probably survived well because they were the survivors of a much larger population and so may have been the most drought resistant portion of the snail colony there. But the over-all results suggest that strain differences among the snails from the different habitats may have been a highly important factor in determining how long the snails lived. The snails that survived least well came from permanent bodies of water in which the snails would have had little opportunity to develop resistance to drying while those surviving best came from localities that dry annually and where the snails would have had opportunity to become adapted to this seasonal stress.

The experiments with *A. glabratus* produced results similar to those obtained with *T. centimetralis*. *A. glabratus* from the lake in Salvador and from the permanent pool in Bairo Novo, survived only a relatively short time in the laboratory without water. However, snails of the same species, collected from temporary pools in Recife and Paulista lived as many as 285 days under essentially the same conditions. Snails collected from the water in Paulista during the rainy season lived about as well out of water as did those collected from the same area during the dry season. When the snails from Salvador and Paulista were compared

the differences in their ability to survive out of water were very obvious. (Figure 2.)

These results with *A. glabratus* strongly suggest that there are marked strain differences with respect to the ability of these snails to survive out of water. Apparently, the Arruda and Paulista snails, living in habitats that dry annually, have acquired the ability to survive out of water while the snails in the other two habitats, which do not dry annually, are less able to survive under these conditions.

It should be emphasized that in no experiment described herein was the relative humidity of the environment very low. Moreover, maintenance of the snails in clay jars tended to keep down air circulation about the snails and so probably provided added protection against rapid water loss. Therefore, the experiments were not tests of the ability of the snails to withstand desiccation but rather tests of their ability to survive out of water at relatively high humidity. Nevertheless,

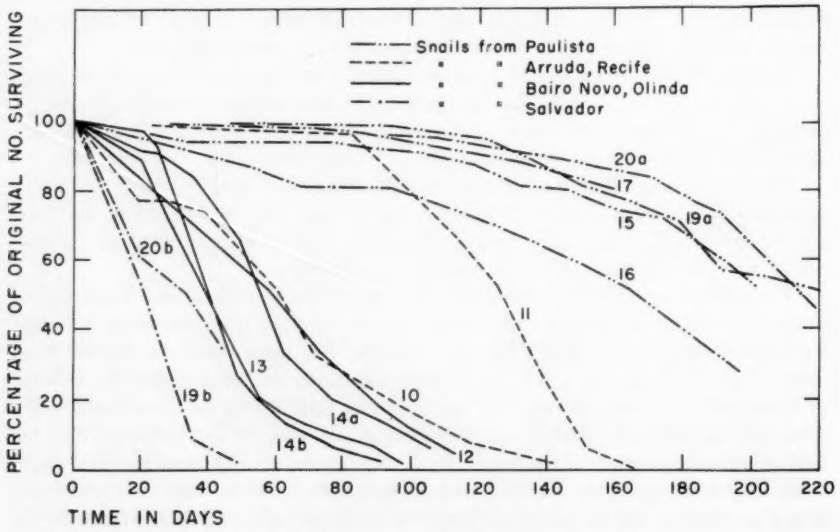

FIG. 2. Survival of *Australorbis glabratus* out of water in clay jars under laboratory conditions.

The numbers on the curves refer to the numbers of the experiments mentioned in the text and in the tables.

Experiments 10 and 11. Snails from the water in Arruda, Recife, a locality which dries annually.

Experiments 12, 13, and 14. Snails from the water in Bairo Novo, Recife, a locality which never dries.

Experiments 15, 16, and 17. Snails collected from a dry habitat in Paulista, a locality which dries annually.

Experiments 19 and 20. Snails from the water in Paulista (a) a locality which dries every year and from a lake in Salvador (b) which never dries.

survival out of water at all but the very highest humidities is accompanied by some water loss, and so even snails at fairly high humidities may lose water and become partially dehydrated if they live for long periods out of water.

The fact that the schistosome vectors of Northeastern Brasil can live out of water in the field for long periods has been demonstrated previously (Barbosa and Dobbin, 1952 and Olivier and Barbosa, 1955a, 1955b). The demonstration that

they can survive for long periods out of water in the laboratory, and that there are probably strain differences with respect to their ability to do this, is new. This does not mean, however, that all *A. glabratus* and *T. centimetralis* from Pernambuco are similarly resistant to drying and the results of experiments 8, 10, and 11 bear this out. For the same reason, one cannot conclude that all *A. glabratus* from Salvador survive poorly out of water. Study of the snails from other localities in Salvador would probably reveal strains with widely varying capabilities for survival out of water.

SUMMARY

Australorbis glabratus and *Tropicorbis centimetralis*, both of which are vectors of schistosomiasis mansoni in Northeastern Brazil, were kept out of water in the laboratory. The snails were maintained without water in shallow, unglazed clay jars. Relative humidities ranged from 70 to 95 percent. There was great variation in the length of time the snails survived but specimens of *A. glabratus* collected from dry habitats lived as long as 207 days and the same species collected from the water lived as many as 285 days. *T. centimetralis* collected from dry habitats lived as many as 14 months and the same species collected from the water to 109 days.

There appear to be important strain differences within both species which determine, in part, their ability to survive out of water. Although some of the snails were able to survive long periods out of water, there was no evidence, in these experiments, that the snails withstand severe desiccation.

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RESEARCH NOTE

THE OCCURRENCE OF AN *ORNITHODOROS* TICK IN MARYLAND

On July 14, 1954, 21 specimens of *Ornithodoros kelleyi* Cooley and Kohls, 1941 were taken in a church in Chaptico, St. Mary's County, Maryland. This is the first record of this species and of the genus *Ornithodoros* in Maryland. *O. kelleyi* was recorded previously from Colorado, Illinois, Iowa, Minnesota, New York, Pennsylvania, Wisconsin and Utah by Cooley and Kohls (1944, Am. Midland Naturalist **1:** 113–117) and from West Virginia by Bequaert (1946, Entom. Am. n. s. **30:** 131–132).

This species has been reported to be strictly an ectoparasite of bats and two larvae of this species were found attached to the neck of two of several *Myotis lucifugus lucifugus* (Le Conte, 1931) taken in this church. The nymphs and adults, however, were all removed from cracks and crevices in the brick wall of the church attic.

In the laboratory the adults and nymphs fed to repletion on *Myotis* in one half to two hours. They fed more readily in a darkened chamber than in the light. Some females oviposited and several hundred larvae hatched from these eggs. Nymphs of the collection, which fed, molted into succeeding nymphal stages or into adults. The larvae, however, did not readily feed on the bats in the laboratory. Only one larva attached and it was forcibly removed in 24 hours after it had distended to several times its original size. This larva subsequently molted into the first nymphal stage and then into the second nymphal stage without another feeding. Further studies on the biology of this species are now in progress.

Three other trips to this same locality resulted in the collection of the following specimens: 9 adults, 2 nymphs, September 25, 1954; 14 adults, 10 nymphs, April 23, 1955; and 76 adults, 43 nymphs, October 15, 1955.

This investigation was supported by a research grant E-738 from the National Microbiological Institute of the National Institutes of Health, Public Health Service.—GEORGE ANASTOS AND CARLETON M. CLIFFORD, *Department of Zoology, University of Maryland.*

STUDIES ON SOME DIPLOSTOME TREMATODES OF THE SUB-FAMILY CRASSIPHIALINI DUBOIS, 1936

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Van Haitsma (1925) created the genus *Crassiphiala* for *C. bulboglossa* Van Haitsma with the following diagnosis:—Diplostome parasites with bisegmented body, vitelline follicles restricted to hind body, acetabulum rudimentary or absent.

Even though *Hemistomum denticulatum* Rudolphi, 1819 had a well developed acetabulum it so closely resembled *C. bulboglossa* in all other respects that Dubois (1933) transferred it to the genus *Crassiphiala*. Hunter (1933), while including under *Crassiphiala* another species, *Hemistomum ambloplitis* Hughes, 1927, a form possessing a well developed acetabulum, amended the generic diagnosis to include forms possessing a well developed acetabulum.

Yamaguti (1934) created the genus *Uvulifer* with *U. gracilis* as the type species. Although *U. gracilis* resembled members of the genus *Crassiphiala*, the new genus differed in the presence of a well developed acetabulum (smaller than the oral sucker), and the presence of a dorso-terminal sucker-like genital atrium which terminated in an uvula-like muscular appendage. At the same time he created the genus *Pseudodiplostomum*, distinguishing it from *Uvulifer* by the fact that the acetabulum was larger than the oral sucker and the uvula-like muscular appendage or genital bulb in the genital atrium was absent. Harwood (1936) amended Hunter's diagnosis of *Crassiphiala* so as to include both *Uvulifer* and *Pseudodiplostomum* as synonyms of *Crassiphiala*, transferring the type species of the two genera to the genus *Crassiphiala*. After a comprehensive study of the group Dubois (1938) came to the conclusion that the presence of a well developed acetabulum whether smaller or larger than the oral sucker is a suitable character for the separation of some genera included under the sub-family *Crassiphialini* Dubois 1936. He, accordingly, recognized the validity of Yamaguti's two genera, *Uvulifer* and *Pseudodiplostomum*. Pande (1938) added a species to the genus *Crassiphiala*, *C. stunkardi*. On closer examination this species is believed to be more closely related to the genus *Uvulifer*, and accordingly is removed to that genus.

The genus *Allodiplostomum* was created by Yamaguti (1935) for *A. scolopacis* Yamaguti, 1935 with the following generic diagnosis: Diplostome parasites with bisegmented body, lateral suctorial cups present on either side of the oral sucker, holdfast organ consisting of three lobes of which two are lateral and one is posteromedian, vitellaria intruding into holdfast organ.

Verma (1936) accepted the genus *Allodiplostomum* and added a new species, *A. hindustani*.

The present paper agrees with Dubois (1953) in retaining the genera *Uvulifer* and *Pseudodiplostomum* as valid genera separate from *Crassiphiala* under the subfamily *Crassiphialini*.

Uvulifer mehrai Chatterji, 1950
(Fig. 1)

Eight specimens of this distome were collected from the small intestine of one of six kingfishers, *Halcyon smyrnensis fusca* taken near Gorukhpur. Measurements are given in millimeters.

Received for publication, September 23, 1955.

Description: Body 0.91–1.58 long. Fore body pear-shaped 0.32–0.46 by 0.28–0.41 in size, with maximum breadth near hold fast organ. Hind body cylindrical, 0.53–1.19 by 0.25–0.36 in size, with maximum breadth near anterior testis. Ratio between length of fore body and hind body roughly 1 : 2. Oral sucker nearly spherical, terminal, with funnel shaped opening, 0.06–0.10 by 0.09–0.12. Pharynx contiguous to oral sucker, 0.04–0.05 by 0.03–0.04. Esophagus 0.03–0.07 long. Intestinal ceca extending nearly to hind end, passing close by holdfast and acetabulum in

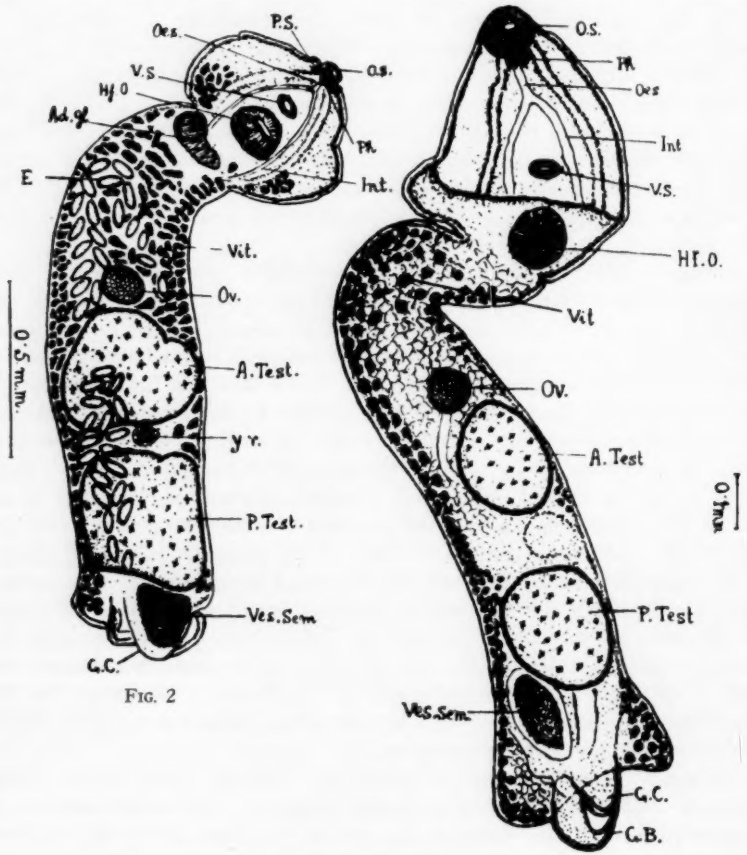

FIG. 2

FIG. 1

EXPLANATION OF FIGURES

FIG. 1. *Uvulifer mehrai.* Chatterji, 1950

FIG. 2. *Allodiplostomum fuscai* Chatterji, 1950

Abbreviations used: Ad. gl., Adhesive gland; A. Test., Anterior testis; E., Egg; G. B., Genital bulb; G. C., Genital cone; Hf. O., Holdfast organ; Int., Intestinal ceca; O. S., Oral sucker; Oes., Esophagus; Ov., Ovary; P. S., Pseudosuckers; Ph., Pharynx; P. Test., Posterior testis; Vit., Vitellaria; Ves. Sem., Vesicula seminalis; V. S., Ventral sucker; Yr., Yolk reservoir.

fore body, mostly obscured by vitelline follicles in hind body. Acetabulum transversely oval, much smaller than oral sucker, 0.03–0.05 by 0.05–0.06, situated 0.05–0.11 behind intestinal bifurcation. Holdfast with a median, longitudinal slit, nearly spherical, 0.07–0.10 by 0.07–0.11, lying close (within 6 to 14 microns) to acetabulum. Gonads tandem, in hind body. Testes postovarian. Anterior testis longitudinally oval, 0.14–0.22 by 0.17–0.21, close behind ovary (14 microns). Posterior testis nearly spherical, 0.21–0.24 by 0.21–0.27, lying 0.04–0.10 behind anterior

testis and 0.17–0.25 in front of hinder end. Vesicula seminalis voluminous, simple, 0.07–0.20 by 0.06–0.10, just behind left border of posterior testis. Genital cone small, dorsal to well developed genital bulb. Genital bulb 0.09–0.14 by 0.10–0.15, protruded in some specimens. Genital opening at hindermost end of body. Ovary nearly spherical, 0.06–0.09 by 0.04–0.10, 0.12–0.28 behind junction of fore and hind body. Receptaculum seminis absent. "Shell gland" mass not seen. Yolk reservoir very small, between testes, a little to right of mid-line. In mounted specimens uterus without eggs. Vitelline follicles aggregated on lateral margins of hind body.

Host: Halcyon smyrnensis fusca (kingfisher).
Habitat: Small intestine.
Locality: Gorukhpur, U. P., India.

Uvulifer mehrai differs from *U. gracilis* Yamaguti, 1934 (type of the genus), *U. prosocotyle* (Lutz, 1928) Dubois, 1937 and *U. denticulatus* (Rudolphi, 1819) Dubois, 1937 in shape and size of the body, shape and size of the gonads, the more posterior disposition of the vitellaria and in having fewer eggs in the uterus. *Uvulifer stunkardi* (Pande, 1938) (syn. *Crassiphiala stunkardi* Pande, 1938), currently being transferred to the genus *Uvulifer,* resembles *U. mehrai* in general topography, and in being almost the same shape and size, as well as in having an acetabulum smaller than the oral sucker, an esophagus, a genital bulb and cone and similar disposition of the vitelline follicles. On the contrary *U. mehrai* differs from that species in that it has differently shaped and larger testes and in having a larger and transversely oval ovary; there is no receptaculum seminis uterinum in *U. mehrai. U. mehrai* differs from *U. errecticus* Chandler and Rausch, 1948 in the presence of a well developed acetabulum, an organ which is very inconspicuous or absent in *U. erecticus,* in the presence of a characteristic genital cone, in different disposition in vitelline follicles and in its occurrence in a different bird host (kingfisher, rather than a passeriform bird).

Allodiplostomum fuscai Chatterji, 1950
(Fig. 2)

Six specimens of the species were collected from the small intestine of one of two king fishers, *Halcyon smyrnensis fusca,* taken from the village Mohoba, district Hamirpur, U. P. Measurements are given in millimeters.

Description: Body 1.37–2.10 long. Cuticle aspinose. Fore body ellipsoid, 0.41–0.45 by 0.39–0.53, with maximum breadth near holdfast. Hind body cylindrical, 0.97–1.65 by 0.34–0.46, with maximum breadth at anterior testis. Ratio of length of fore body to hind body roughly 1 : 2.5. Oral sucker small, terminal, spherical, 0.03–0.05 by 0.04–0.06. Pseudosuckers present, adjacent to oral sucker, 0.04 by 0.03. Pharynx small, contiguous with oral sucker, 0.03–0.04 by 0.03. Esophagus small, 0.03–0.04 long. Intestinal ceca laterally on either side of holdfast and acetabulum, superimposed by vitelline follicles in hind body. Acetabulum small, transversely oval, 0.02–0.05 by 0.05–0.08, situated 0.05–0.07 behind intestinal bifurcation. Holdfast with narrow, transverse slit, transversely oval, 0.13–0.18 by 0.13–0.18, situated very close to acetabulum or 0.03 behind it. In sections holdfast with two outgrowths, characteristic of the genus *Allodiplostomum.* Adhesive gland (tribocytic gland) small, at anteriormost end of hind body. Testes tandem, post-ovarian, occupying almost entire width of hind body; anterior testis pear-shaped, 0.20–0.35 by 0.38–0.41; posterior testis more or less rhomboid, 0.24–0.38 by 0.31–0.39, 0.21–0.35 in front of hinder end. Vesicula seminalis simple, situated close behind posterior testis. Ovary pretesticular, almost spherical, 0.07–0.11 by 0.09–0.13, 0.25–0.45 behind junction of fore and hind body. Yolk reservoir small, between testes. Hermaphroditic canal small, traversing well developed genital cone. Genital bulb well developed. Eggs non-operculated (apparently), yellowish brown in color, longitudinally oval, 0.04–0.08 by 0.03–0.05. Vitelline follicles small, extending throughout length and breadth of hind body, and some follicles extending into fore body to level of holdfast.

Host: Halcyon smyrnensis fusca. (kingfisher).
Habitat: Small intestine.
Locality: Mohoba (Hamirpur), U. P. India.

Allodiplostomum fuscai comes close to the type species, *A. scolopacis* Yamaguti, 1935, in general topography, presence of pseudosuckers, and slight extension of

vitellaria into the fore body. However it differs from that species in shape and size of the fore body, shape and size of the anterior testis, shape and size of the vesicula seminalis and in the presence of a small adhesive gland. The species resembles *A. hindustani* Verma, 1936 in general topography, presence of pseudosuckers, lobed condition of the holdfast, slight extension of the vitellaria into the fore body, but differs from it in its larger size and different shape of body, shape and size of testes, shape of vesicula seminalis and in the larger number of eggs in the uterus.

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RESEARCH NOTE

A PRACTICAL METHOD FOR RAISING LAMBS "PARASITE FREE" WHILE ALLOWING THEM TO REMAIN WITH THEIR EWES

Prohibitive cost of labor and management as well as questionable nutritional conditions, prevent most investigators of sheep parasites from raising lambs on wire, away from their ewes. The following method is used by the authors and is apparently effective in keeping lambs free of coccidia and nematodes except *Strongyloides papillosus.*

One month before lambing, all ewes are drenched with 25 grams of phenothiazine and trimmed to remove all soiled wool. They are then fed a pelleted ration consisting of ground alfalfa hay, ground yellow corn, molasses, one percent trace mineralized salt, and enough phenothiazine so that each ewe receives between 1.5 and 2 grams per day. Our practice is to have four pounds of phenothiazine mixed in one ton of feed. One pint of a 3.45 percent solution of sulfaquinoxaline is added to 50 gallons of drinking water. Both drugs are administered continuously until the lambs are ready for experimental use. While using this method we have used straw bedding in concrete pens. The pens are routinely cleaned twice weekly.

The method is apparently effective. From critical observations while doing daily routine fecal examinations, coccidia oocysts have not appeared for a period of eighteen days following discontinuation of the preventive measures and no unwanted nematode infections (except *Strongyloides*) have been encountered. No drug toxicity or abortions have been noticed.—R. F. Shumard and D. F. Eveleth, *Department of Veterinary Science, North Dakota Agricultural Experiment Station, Fargo.* (Published with approval of the Director of the North Dakota Agricultural Experiment Station.)

TREMATODES OF FISHES FROM THE RED SEA. PART 6. ON FIVE DISTOMES INCLUDING ONE NEW GENUS AND FOUR NEW SPECIES*

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Part 6 in this series includes descriptions of 5 trematodes of which one is recorded for the first time from a fish occurring in the Red Sea and 4 are new species, one representing a new genus. The author collected this material in Egypt and completed its study at the University of Nebraska during the tenure of a Fulbright Scholarship Award for 1953–54. Type specimens are kept at the Parasitology Department, Faculty of Medicine, Abbasia, Cairo, Egypt. All measurements are in mm.

FAMILY OPECOELIDAE

Helicometra hypoditis Yamaguti, 1934
(Fig. 1)

Two mature specimens were collected from 4 specimens of *Serranus* (=*Epinephalus*) sp., locally called "Koshar," from Ghardaga.

Diagnosis: Body tapering anteriorly, broadly rounded posteriorly; 2.16 and 2.03 long, 1.21 and 1.01 maximum width. Acetabulum 0.33 in diameter, near anterior third of body. Oral sucker 0.21 by 0.25 in diameter; forebody 0.33 long. Prepharynx short; pharynx 0.98 long by 0.14 wide; esophagus short; intestinal ceca ending 0.2 from posterior end of body. Testes tandem, lobed, wider than long, 0.29 by 0.52 to 0.59; in posterior half of body. Cirrus sac curved in an S-shape, extending from middle of acetabulum to genital atrium near pharynx. Genital pore median, ventral to pharynx. Ovary directly anterior to testes; wider than long; lobed; size 0.16 by 0.24. Seminal receptacle large, pyriform, immediately anterior and to right of ovary. Vitellaria mostly extracecal, from level of pharynx to near posterior end of body. Uterus between ovary and acetabulum; eggs yellowish, thin-shelled with long filament about twice as long as egg shell which measures 0.04 to 0.06 by 0.03 to 0.04.

This species is previously known from *Hypodytis rubipinnis* in the Inland Sea, Japan.

FAMILY SPHINCTEROSTOMATIDAE

Megacreadium tetrodontis n. g., n. sp.
(Fig. 2)

Only one mature specimen was obtained from the alimentary tract of *Tetrodon* sp. from Ghardaga.

Diagnosis: Length 13.5, maximum width 4.7; with almost parallel sides; cuticle smooth. Oral sucker large and globular, 2. in diameter, highly cellular. Surrounding mouth are 2 large muscular, lateral lobes and one smaller, median, posterior lobe, each scalloped or frilled with short processes. Acetabulum salient, smaller than oral sucker; 0.7 in diameter; at junction of 1st and 2nd quarters of body length. Sucker ratio 1: 0.35. Pharynx poorly developed, with circular muscles only; measuring 0.5 long by 0.84 wide. Prepharynx and esophagus lacking. Ceca broad, opening posteriorly through ani.

Testes 8, entire, more or less globular, 0.4 to 0.6 in diameter, in two irregular, longitudinal rows of 3 on the left and 5 on the right, intercecal, slightly anterior to middle of body. Cirrus sac absent; seminal vesicle an elongate sac measuring 1. by 0.6 in diameter, situated to right of and slightly anterior to acetabulum; prostatic cells free in parenchyma. Genital pore submedian, very slightly to the left, immediately anterior to acetabulum. Ovary spheroid, entire, pretesticular, of about same size as testes, slightly to right of midline, near end of anterior third of body length. Shell gland complex to left of ovary closely posterior to acetabulum. Seminal receptacle not observed. Vitelline follicles comparatively small and numerous, extending from posterior end of body almost to ovary. Uterus compact, to left of midline, extending from slightly posterior to acetabulum to pharynx, so that approximately half of it is anterior to genital pore. A short metraterm leads posteriorly to genital pore. Eggs yellowish, ovoid, large, measuring 0.14 by 0.09; shell without filaments or knobs.

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* Studies from the Department of Zoology, University of Nebraska, No. 284.

Generic diagnosis of Megacreadium. Fairly large, unspined trematodes; oral sucker very large, highly cellular, with two lateral and one posterior muscular lobes around mouth; pharynx weakly muscular; two ani present. Testes 8, in two more or less longitudinal rows of 5 and 3, in middle third of body; saccular seminal vesicle; no cirrus sac; prostatic cells free in parenchyma. Ovary pretesticular, entire; seminal receptacle not observed; vitellaria of small follicles, extending from near posterior border of ovary to posterior end of body; uterus to left of acetabulum, extending anterior to genital pore. Genital pore submedian, preacetabular. Eggs large, without filaments. Parasites of marine fishes. Type species: *M. tetrodontis.*

Discussion: Except for the 8 testes, *Megacreadium* has some similarity to the genus *Sphincterostoma* Yamaguti, 1937, for example in its cellular oral sucker, sucker ratio, and lack of cirrus sac. *Sphincterostoma* also has muscular preoral lobes, but it differs in having two testes and its ceca join the excretory vesicle. The number of testes and the presence of ani are generic characters in both the Lepocreadiidae and the Opecoelidae. It might be noted that the status of the family Sphincterostomatidae is rather uncertain. Yamaguti established it in 1937 and it still contains only the single species, *Sphincterostoma branchiostegi.* Later, Yamaguti (1954) included it, along with many genera of Opecoelidae and Lepocreadiidae, in the Allocreadiidae. It is recognized here tentatively pending knowledge of more species or life cycles.

FAMILY GORGORDERIDAE

Phyllodistomum leilae n. sp.
(Fig. 3)

One specimen was obtained from the intestine of *Pseudoscarus harid,* locally known as "harid," from Ghardaga.

Diagnosis: Body tapering toward both ends, not abruptly broadened; 6.3 long by 1.83 in maximum width. Cuticle transversly striated. Acetabulum at junction of 1st and 2nd fourths of body length, 0.44 in diameter. Oral sucker 0.5 in diameter; esophagus very short and narrow, glandular; pharynx lacking; ceca with numerous indentations, ending blindly near posterior end of body; right cecum somewhat longer than the left.

Testes fairly large, somewhat longer than wide, indented, intercecal, in middle 3rd of body length, oblique, right testis in advance of left, overlapping each other by about ¼ length. Seminal vesicle ovoid, median, entirely anterior to acetabulum. Ovary ovoid, entire, pretesticular, to left of midline, partly overlapping left cecum, at junction of anterior and middle 3rds of body length. Vitellaria of two masses, each composed of 7–9 lobes, mainly intercecal, closely anterior to ovary. Seminal receptacle posterior to ovary. Uterus filling most of intercecal space posterior to testes, extending almost as far back as posterior end of right cecum, then anteriorly between left testis and ovary, to right of ovary, between vitellaria to genital pore midway between suckers. No eggs in uterus. Excretory vesicle narrow, extending to posterior border of ovary before bifurcating.

Discussion: This species is characterized by its very short esophagus, irregularly constricted ceca, and a sucker ratio of 1:0.88. It is perhaps most similar to *P. pacificum* Yamaguti, 1951 from *Caranx equula* in Japan, but differs in more lobed vitellaria, less lobed ovary, longer posttesticular distance, and constricted ceca.

FAMILY HEMIURIDAE

Hysterolecitha teuthis n. sp.
(Fig. 4)

Only one specimen was obtained from the alimentary canal of *Teuthis marmorata,* locally called "Sigan," from Ghardaga.

Diagnosis: Body elongated, with parallel sides; length 3.25; width 0.73; both ends broadly rounded, slight indentation at excretory pore; cuticle smooth. Acetabulum 0.44 in diameter, occupying most of 2nd fourth of body length. Oral sucker 0.29 in diameter; sucker ratio about 1: 1.5. Pharynx small; no prepharynx or esophagus; ceca simple, broad, with shoulders anteriorly, a constriction on each side opposite anterior half of acetabulum, and a peculiar fold opposite posterior half of actabulum. Ceca end blindly at posterior end of body.

Testes two, oblique, ovoid, entire, immediately posterior to acetabulum; seminal vesicle free

in parenchyma, sinuous, extending from anterior border of acetabulum to about ½ distance between acetabulum and intestinal bifurcation; pars prostatica well developed, surrounded by prostatic cells; genital atrium sucker-like, a short distance posterior to bifurcation. Ovary ovoid, about same size as testes, median, at anterior end of posterior 3rd of body length. Vitellaria immediately postovarian, composed of 7 digitiform processes directed laterally and posteriorly. Uterus filling most of body posterior to acetabulum, separated testes from ovary and extending posterior to ovary and vitellarium; eggs 0.036 by 0.021.

Discussion: H. rosea Linton, 1910, type of the genus, is from *Teuthis* (= *Acanthurus*) at Tortugas, Florida. *H. acanthuri* Annereaux, 1947 is also from that genus of fish. *H. teuthis,* however, is most similar to *H. lintoni* Srivastava, 1939 from *Arius dussumieri* in the Arabian Sea, agreeing in size, sucker ratio, broad prostatic gland, and location of gonads. The chief difference is in egg size, 0.036 by 0.021 in *H. teuthis* as compared with 0.023 to 0.027 by 0.0076 to 0.010 in *H. lintoni.* It is also very similar to *H. nahaensis* Yamaguti, 1942 which, however, has a sucker ratio of 1 : 1.6 to 2.7, somewhat more anterior acetabulum and ovary, and eggs 0.021 to 0.027 by 0.009 to 0.012. It differs from *H. microrchis* Yamaguti, 1934 chiefly in sucker ratio and egg size.

Tubulovesicula serrani n. sp.
(Fig. 5)

Only one specimen was obtained from the alimentary canal of *Serranus* sp., locally called "Koshar," from Ghardaga.

Diagnosis: Body more or less fusiform with very long, slender ecsoma equal in length to that of body proper; about ½ of ecsoma extended; total length of trematode 4.5; maximum width 0.8; body length 3.2. Acetabulum wider than long, 0.5 by 0.44; forebody 0.8 long. Oral sucker subterminal, 0.23 in diameter; sucker ratio 1 : 2.17; pharynx wider than long, 0.123 by 0.105; no prepharynx or esophagus; two kidney-shaped cecal swellings, one on either side of pharynx; ceca simple, unequal in length, ending blindly; right cecum almost reaching posterior end of ecsoma; left cecum about 0.4 shorter.

Testes spheroid, slightly oblique, close to postero-lateral border of acetabulum and partly overlapped by it, at junction of 1st and 2nd fourths of body length; seminal vesicle sinuous, dorsal to posterior half of acetabulum; pars prostatica surrounded by prostatic cells, beginning slightly anterior to midacetabular level; from a ventral view the length of pars prostatica appears to be slightly less than length of seminal vesicle; sinus sac ovoid, thick-walled, containing a hermaphroditic vesicle and hermaphroditic duct; genital pore immediately anterior to intestinal bifurcation. Ovary ovoid, almost twice as large as one testis, to right of midline, posterior to right testis and overlapped dorsally by right cecum. Vitellaria composed of 8 tubes, 4 on each side, just posterior to ovary, extending lateral to ceca; uterus between ovary and ecsoma, extending lateral to ceca, entering only slightly base of ecsoma; eggs ovoid, thick-shelled, 0.027 by 0.020.

Discussion: Manter (1954) has shown that the number of vitelline tubes in a species of *Tubulovesicula* may be either 7 or 8 arranged in various positions, *T. serrani* is very similar to *T. anguillae* Yamaguti, 1934 which also has a very long ecsoma (equal to body length). The chief difference is the very long pars prostatica in *T. anguillae* where it is much longer than the seminal vesicle; also, in *T. anguillae,* the uterus extends rather far into the ecsoma and eggs are 0.034 by 0.023 to 0.024. *T. serrani* differs from *T. angusticauda* (Nicoll, 1915) in much longer ecsoma and more posterior extent of the pars prostatica.

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YAMAGUTI, SATYU 1937 Studies on the helminth fauna of Japan. Part 17. Trematodes from a marine fish, *Branchiostegus japonicus* (Houttuyn). 15 pp. Pub. by author.

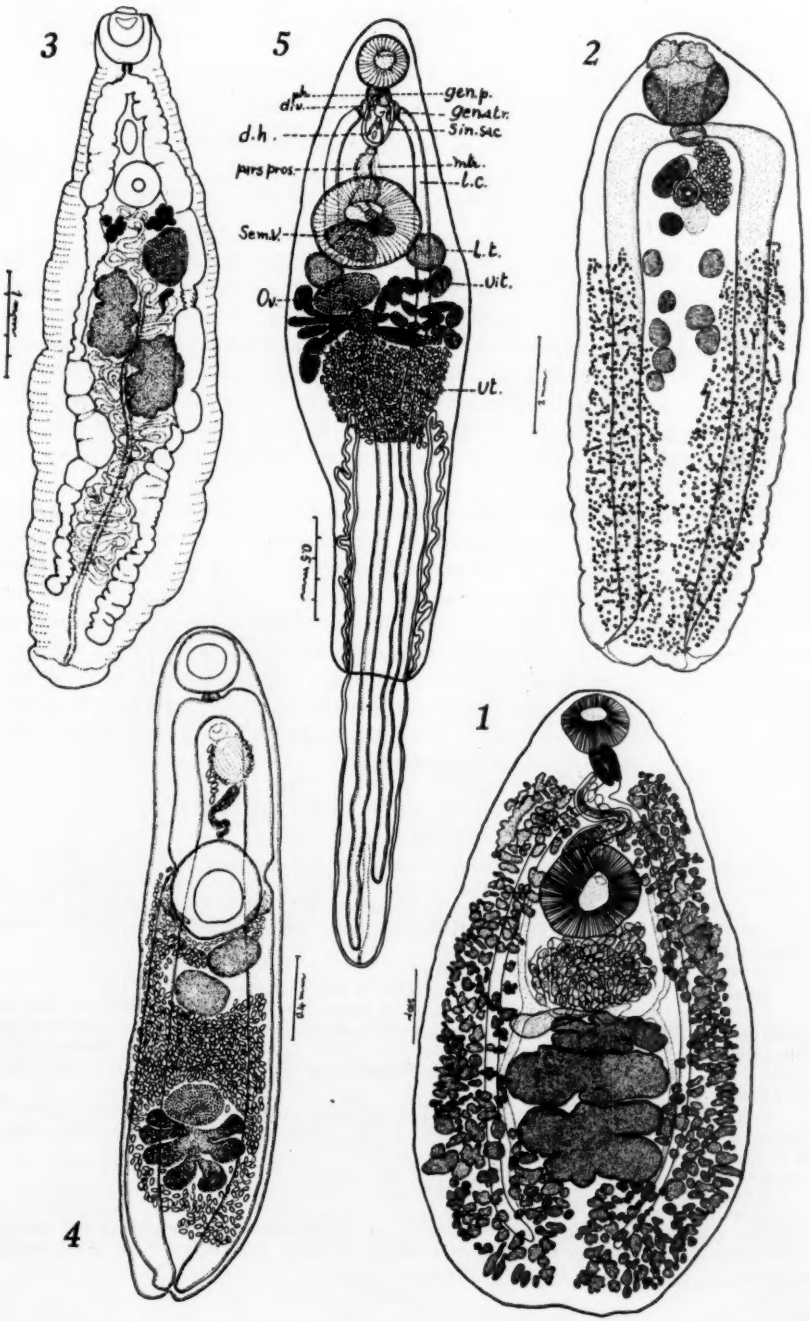
———1953 Systema Helminthum Part I. Digenetic trematodes of fishes. Maruzen Co., Ltd., Tokyo, Japan.

Explanation of Plate

Fig. 1. *Helicometra hypoditis* Yamaguti, 1934. Ventral view.
Fig. 2. *Megacreadium tetrodontis.* Ventral view.
Fig. 3. *Phyllodistomum leilae.* Ventral view.
Fig. 4. *Hysterolecitha teuthis.* Ventral view.
Fig. 5. *Tubulovesicula serrani.* Ventral view.

Abbreviations used: *div,* cecal swelling; *d.h.,* ductus hermaphroditicus; *gen.atr.,* genital atrium; *l.t.,* left testis; *mtr.,* metraterm; *ov.,* ovary; *pars pros.,* pars prostatica; *ph.,* pharynx; *sem.v.,* seminal vesicle; *sin.sac,* sinus sac; *vit.,* vitelline tube; *ut.,* uterus.

PLATE I



NOTES ON AFRICAN *HAEMAPHYSALIS* TICKS. III. THE HYRAX PARASITES, *H. BEQUAERTI* SP. NOV., *H. ORIENTALIS* N. AND W., 1915 (NEW COMBINATION), AND *H. COOLEYI* BEDFORD, 1929 (IXODOIDEA, IXODIDAE)

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Hyrax-parasitizing *Haemaphysalis* ticks are most difficult to obtain and are rare in collections. In my encounters with hyraxes in East Africa, North Africa, Sinai, and Arabia, only a few East African hosts have been found infested. It seems probable that these ticks feed for but short periods. When off the host they are impossible to secure from deep within narrow clefts on steep mountainsides where hyraxes make their home. Thus, it can hardly be determined whether actual rarity in nature or short-term feeding accounts for the paucity of available specimens. In part, the infrequency with which collectors interested in ticks see and examine hyraxes is a factor.

The three haemaphysalid species herein discussed form a complex allied to the carnivore-parasitizing *H. leachii* group of Africa, Asia, and related archipelagoes and to the *H. hoodi* group, a bird-parasitizing complex of more localized species within the same area. For convenience, we shall refer to the hyrax parasites as the "*H. orientalis* group". The tailless, rodentlike hosts, among the oldest mammals in Africa are actually small, herbivorous ungulates with rhinoceros-like teeth and with feet indicating their relationship to the elephants. The geographical range of hyraxes includes much of tropical and southern Africa, parts of northern Africa, also Sinai, Arabia, and the Near East. Their haemaphysalid parasites, however, are known only from eastern and southern Africa.

HAEMAPHYSALIS BEQUAERTI SP. NOV.

(Figures 1 to 23)

H(aemaphysalis) sp. nov. Hoogstraal, 1954A, p. 307, note on presence in Sudan fauna.

Holotype: Male, from a female rock hyrax, *Heterohyrax brucei hoogstraali* Setzer, (in press) (HH 5130), Imurok, Torit District, Equatoria Province, (Anglo-Egyptian) Sudan; 2500 feet elevation, 8 February 1950, H. Hoogstraal *legit.* Deposited in United States National Museum, Number 2216.

Allotype: Female, same data as holotype.

Paratypes: Total: one larva, thirteen nymphs, 74 males, 23 females.

From *Hoogstraal collection:* One nymph, five males, two females, same collecting data as holotype. Two males, same collecting data as holotype except that the immature host (HH 5110) was taken on 1 February 1950. Seven males from *Procavia habessinica slatini* Thomas, 1907 (HH 3756), Lui, Yambio District, Equatoria Province, (Anglo-Egyptian) Sudan, 1 May 1948, R. Alison *legit.*

From *British Museum (Natural History):* Four males and three females: "1929.10.10.34–41. Naishi, Kenya, from hyrax, May 5, 1929.32. P. M. Jenkin (c). Joint Exped. Brit. Assoc. & P. Sladen Trust to Rift Valley Lakes, Kenya, 1929."

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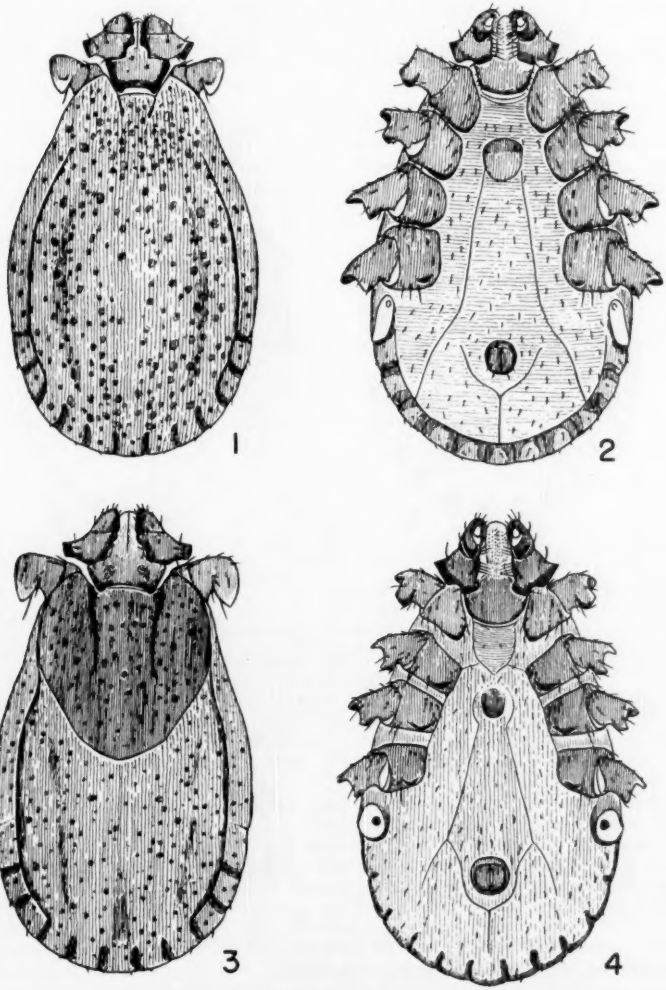
Research Report NM 005 050.29.24

The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

From *Onderstepoort collection:* 56 males, 18 females, 12 nymphs, 1 larva, lot numbers 2454 and 2974: from several specimens of *Procavia capensis meneliki* Neumann, 1902, at Kaabong, Karamoja, Uganda, various dates from 1952 to 1955, A. J. Haddow *legit.*



EXPLANATION OF FIGURES

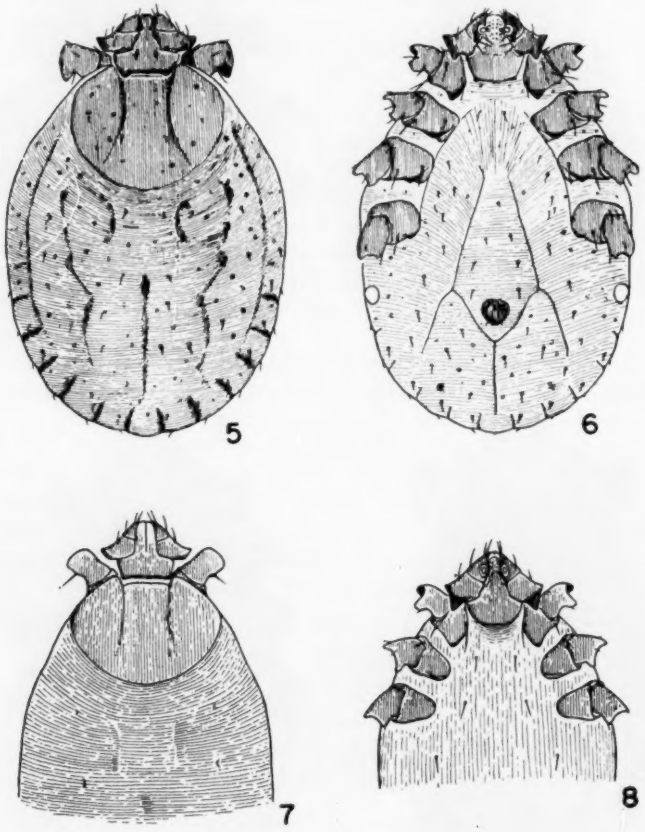
Haemaphysalis bequaerti sp. nov. (Torit District, Sudan, Paratypes).
MALE: FIG. 1, dorsal view. FIG. 2, ventral view.
FEMALE: FIG. 3, dorsal view. FIG. 4, ventral view.

The British Museum (Natural History) specimens and most of the Onderstepoort series are deposited in their respective collections. Other paratypes are deposited in collections of the Rocky Mountain Laboratory, United States National Museum, Chicago Natural History Museum, Museum of Comparative Zoölogy, East African Veterinary Research Organization, and the writer.

DESCRIPTION. MALE: *Length* overall, from 1.66 mm. to 1.88 mm., maximum *width*

from 0.99 mm. to 1.22 mm. *Body* elongate oval, about three-fifths as wide as long, widest about midlength, posterior margin broadly rounded, lateral margins convex, anteriorly more or less abruptly converging to scapulae (small specimens are frequently wider in outline than large ones). *Color* pale yellow to brownish or reddish yellow, legs pale yellow.

Capitulum: Basis capituli apically almost twice as wide as long (including cornua); lateral margins strongly diverging anteriorly; surface impunctate or with two to four shallow punctations; posterior margin between cornua very slightly concave; cornua about one fourth as long



Haemaphysalis bequaerti sp. nov. (Uganda paratypes).

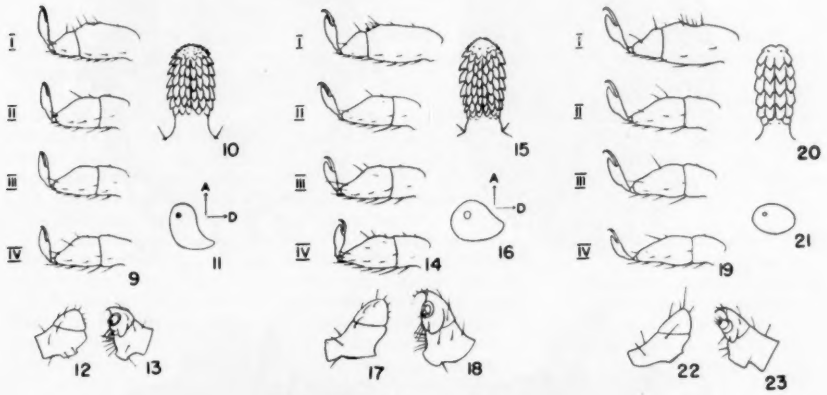
NYMPH: FIG. 5, dorsal view. FIG. 6, ventral view.
LARVA (greatly engorged): FIG. 7, dorsal view. FIG. 8, ventral view.

as basis capituli, forming a bluntly pointed, usually wide triangle. *Palpi* short and subbasally salient; 2.3 times as wide as long; dorsally basal margin slightly concave between insertion and more or less weakly produced basolateral angle; lateral margin basally forming an acute angle from tip of basal juncture to about midlength of segment 2, thence acutely recurved to base of segment 3; segment 3 gradually narrowing, bluntly rounded or truncate apically, about one half as long as segment 2; palpal hairs as illustrated. *Palpi ventrally* with outline as from dorsal view except that the concavity between insertion and basolateral angle is usually slightly more emarginate and the basolateral juncture may be more pointed; segment 3 about one half as long as segment 2, with a very short, broad, rounded, retrograde spur which usually, but not always, slightly overlaps the apical margin of segment 2. *Hypostome* stoutly elongate, broadly

rounded anteriorly with weak corona; length about 110 microns; dentition 4/4; denticles robust, overlapping, in files of six or seven occupying the anterior two-thirds of the hypostome.

Scutum: Lateral grooves fairly deep and narrow, extending to anterior fourth of scutum, posteriorly enclosing first and second pairs of festoons (extension bordering the second festoon sometimes shallow or obscure). *Cervical pits* short, deep, converging; *cervical grooves* faint to obsolete, diverging, may extend to apical level of lateral grooves. *Festoons* clearly marked, mostly slightly wider than long. *Scutal surface* somewhat convex. *Punctations* only moderately numerous; distributed unevenly over entire surface including festoons and lateral areas, usually fewer in central area and slightly more numerous on anterior fourth of scutum than elsewhere; shallow, coarse, nondiscrete, mostly large, some medium size; mostly widely separated but some adjacent where they tend to run in lines.

Legs: Coxae weakly armed; spurs in the form of slight ridges hardly if at all reaching posterior margin; I broadly subtriangular, basally bluntly pointed; other coxae subrectangular; II and III with a basal, very slightly raised, inconspicuous ridge which in II may be pointed at



Haemaphysalis bequaerti sp. nov.

MALE: FIG. 9, tarsi I to IV, lateral view. FIG. 10, hypostome, ventral view. FIG. 11, spiracular plate. FIG. 12, palpus, dorsal view. FIG. 13, palpus, ventral view.

FEMALE: FIG. 14, tarsi I to IV, lateral view. FIG. 15, hypostome, ventral view. FIG. 16, spiracular plate. FIG. 17, palpus, dorsal view. FIG. 18, palpus, ventral view.

NYMPH: FIG. 19, tarsi I to IV, lateral view. FIG. 20, hypostome, ventral view. FIG. 21, spiracular plate. FIG. 22, palpus, dorsal view. FIG. 23, palpus, ventral view.

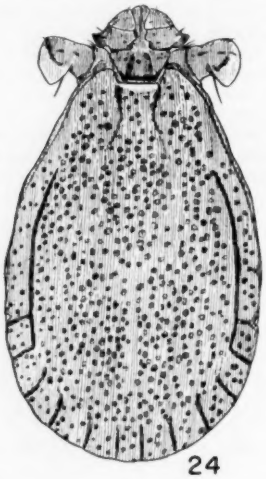
its inner margin; IV with a faint ridge or none; coxae with maximum of six small, white, inconspicuous hairs shorter than coxae. *Trochanters* unarmed ventrally; I dorsally with a moderately large, triangular shield. *Tarsi* moderate; I very slightly longer than wide and gradually tapering more or less from base; other tarsi subequal, about two-thirds as wide as long, dorsal and ventral margins parallel basally, apically tapering gradually from midlength to apex (in small specimens tarsal tapering may be more abrupt or more concave in lateral aspect). *Pads* and *claws* moderate, pads reaching almost to apex of claws.

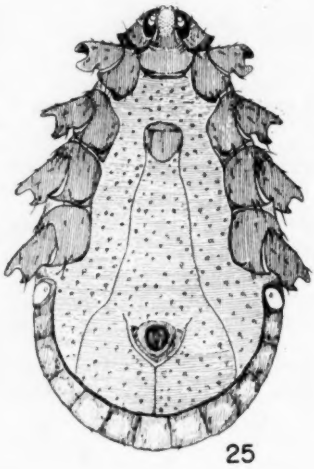
Ventral surface: Genital aperture situated between coxae II, wide, with broadly rounded anterior margin and more elongate and narrowly rounded posterior margin. *Genital* and *anal grooves* distinct, as illustrated. *Spiracular plate* small, transversely elongate, with a small bluntly rounded dorsal projection.

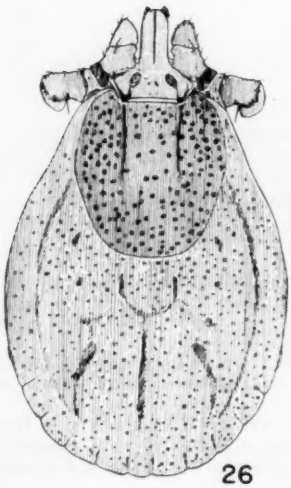
FEMALE: This sex is associated with males by similarity of critical characters; differences are chiefly in secondary sexual characters. *Length* and *width* of unengorged specimens in most series is only very slightly greater than that of male, in other series measuring up to half again as large as associated males. *Body outline* similar to that of males, upon engorgement becoming somewhat wider posterior of scutum.

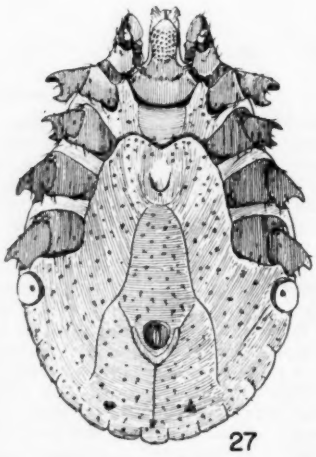
Capitulum: Basis capituli short and wide, 2.5 times as wide as long; lateral margins strongly diverging anteriorly; posterior margin slightly concave, less commonly straight; cornua small, triangular with bluntly rounded apex, in some specimens reduced to even smaller, rounded projections; surface impunctate, porose areas shallow, tilted, oval. *Palpi* similar to those of male except that they are more elongate and the ventral retrograde spur of segment 3 is even shorter,

usually not overlapping the apical margin of segment 2. *Hypostome* similar to that of male except that it is slightly more elongate, the apex is slightly more extended, denticles are in files of seven to nine and occupy about four-fifths of the hypostomal length, and outer files are proportionately slightly more massive than inner files.









Haemaphysalis orientalis (Cotypes).

MALE: FIG. 24, dorsal view. FIG. 25, ventral view.
FEMALE: FIG. 26, dorsal view. FIG. 27, ventral view.

Scutum: Very slightly longer than wide; scapulae bluntly rounded; lateral margins widely divergent from scapulae to anterior sixth of scutal length, thence slightly divergent to midlength, posteriorly rather abruptly narrowed and pointed. *Cervical grooves* varying from shallow to deep; extending to posterior third of scutum; slightly convergent to scutal midlength, thence divergent. *Punctations* indistinct, shallow, large and medium size, few, maximum number about forty.

Legs: Coxae narrower than those of male but with similar spurs and ridges, which in some specimens are more darkened and therefore more conspicuous; some coxal hairs longer than in male. *Pads* very slightly shorter in relation to claws than in male. Characters otherwise as in male.

Spiracular plate subcircular with a small, short, wide, bluntly rounded dorsal projection.

NYMPH: The nymph differs from adults in certain significant respects (by which its close relationship to *H. leachii* is shown).

Capitulum: Palpi have either a short, compact outline (as illustrated) similar to that of the male or a more elongate outline similar to that of the female; but the dorsal basolateral juncture is less acutely pointed while (*notably*) the ventral basolateral juncture forms a spurlike angle. The ventral retrograde spur of palpal segment 3 is comparatively narrow and its narrowed, bluntly rounded apex slightly overlaps the basal margin of segment 3. *Basis capituli* short and wide, at least two and a half times as wide as long; cornua short, triangular, apically blunt projections as in the female or even more reduced. *Hypostome* with large denticles, apex slightly indented; dentition 2/2; denticles large, in files of six or seven.

Scutum: Outline short and wide, about two-thirds as long as wide, with rounded margins having a subcircular appearance. *Cervical grooves* converge for almost half their length and thence diverge widely, reaching almost to posterior scutal margin. *Punctations* indistinct, shallow and sparse, numbering fewer than 15.

Legs: Coxae comparable with those of female in shape but spurs more pointed and those on II and III very slightly overlap the basal margin of coxa (coxal spurs may be difficult to distinguish in pale specimens). *Trochanter* I with moderate, triangular shield dorsally. *Tarsi* similar to those of the adult except that the dorsal narrowing of I is more concave and IV is slightly more elongate and gradually narrowed. *Pads* and *claws* like those of adults except that pads are slightly shorter in relation to length of their respective claws.

Spiracular plate small, ovoid, lacking dorsal projection.

LARVA: The single, greatly engorged available specimen, collected from the same host with nymphs and adults, is broken across the middle of the body and lacks legs. It differs from any other known haemaphysalid larva of this area and is most probably the species under discussion.

Capitulum: Palpi with outlines similar to that of nymph except more elongate, segment 3 almost as long as segment 2; basolateral spur very pronounced and no apparent retrograde spur on ventral surface of segment 3 (? damaged). *Basis capituli* three times as wide as long; impunctate; lateral margins moderately diverging anteriorly; basal margin very slightly concave; cornua lacking. *Hypostome* features indiscernible in this specimen.

Scutum: Outline ovoid, two-thirds as long as wide; apparently impunctate; cervical grooves almost parallel, extending to scutal midlength, thence diverging but shallow and vague.

Legs: Trochanter I apparently lacking a dorsal shield (? broken). *Coxa* I subtriangular, pointed basally; II subrectangular with rounded inner margin and very slight basal ridge; III with somewhat more narrowly rounded inner margin than II, lacking either ridge or spur.

RELATED SPECIES: See below.

HAEMAPHYSALIS ORIENTALIS NUTTALL AND WARBURTON, comb. nov.

(Figures 24 to 36)

Haemaphysalis hoodi var. *orientalis* Nuttall and Warburton, 1915, pp. 486–487, male and female briefly and inadequately described without illustrations, from six males and three females from *Heterohyrax brucei manningi* (Wroughton, 1910) (syn. *Procavia manningi*) from the southwest shore of Lake Nyassa, Nyasaland (= B.E.A.), March, 1910. S. A. Neave *legit.* Types in British Museum (Natural History) (Nuttall lots 2847 and 2848; original numbers 66a and 69).

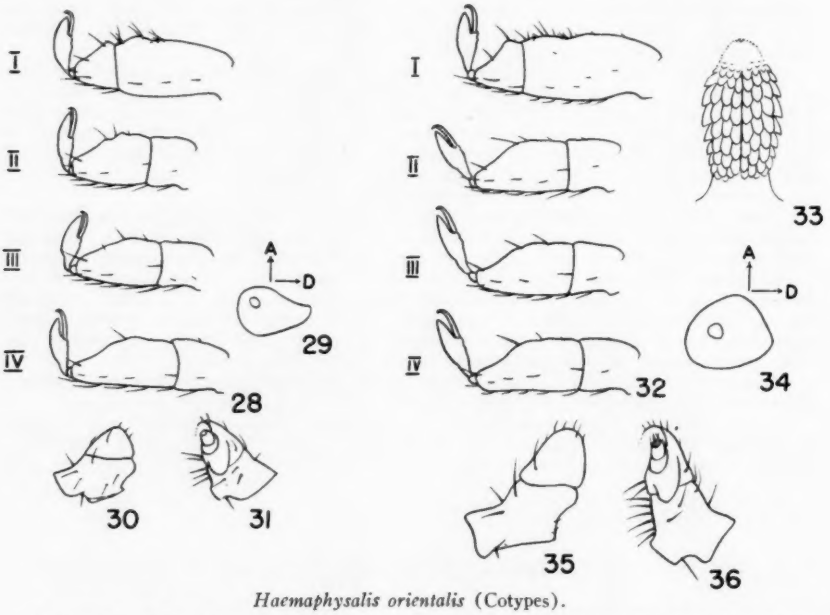
Haemaphysalis zambeziae Santos Dias, 1954A, pp. 1–7, description of single male and two females [compared with *H. erinacei* Pavesi, 1884 (syn. *H. numidiana* Neumann, 1905)]. For remarks on the host, see "*Hosts and host-parasite relationships*" below.

Material examined: Complete Nuttall and Warburton cotype series except that one female is now missing.

REDESCRIPTION. MALE: *Length* overall, 1.947 mm., greatest *width* 1.089 mm. *Body* oval, about two-thirds as wide as long; widest just posterior of midlength; posterior margin broadly rounded from level of anterior festoons; lateral margins almost straight, gradually narrowing from midlength to anterior fourth of scutal length, thence acutely narrowing to scapulae.

Capitulum: Basis capituli very strongly diverging anteriorly, posterior margin straight and with only minute cornua which are all but obsolete (hardly, though, "without cornua," as stated in original description), surface with several shallow punctations and slightly depressed centrally. *Palpi* very short and subbasally salient; 2.45 times as wide as long; dorsally basal margin slightly concave between insertion and bluntly rounded basolateral angle; lateral margin basally forming an acute angle from rounded basal juncture to about midlength of segment 2; thence acutely recurved, as a very slightly upturned point or as a rounded juncture (variation from side to side in specimen at hand), in a slightly concave line to base of segment 3; segment 3 triangular, bluntly pointed apically; segments 2 and 3 of almost equal length. *Palpi ventrally* with outline as from dorsal view except that basolateral juncture is even more rounded; segment 3 with a broad, rounded retrograde spur slightly overlapping the apical margin of segment 2. *Hypostome* (single mount not clear) dentition 4/4, files of apparently six to eight denticles.

Scutum: Length (five specimens) from 1.65 mm. to 1.74 mm., *width* 1.00 mm. to 1.20 mm. (Nuttall's measurements). *Lateral grooves* deep and narrow, extending to anterior fourth of scutum, posteriorly enclosing first pair of festoons. *Cervical pits* short, deep, converging,



Haemaphysalis orientalis (Cotypes).

MALE: FIG. 28, tarsi I to IV, lateral view. FIG. 29, spiracular plate. FIG. 30, palpus, dorsal view. FIG. 31, palpus, ventral view.

FEMALE: FIG. 32, tarsi I to IV, lateral view. FIG. 33, hypostome, ventral view. FIG. 34, spiracular plate. FIG. 35, palpus, dorsal view. FIG. 36, palpus, ventral view.

cervical grooves shallow, basally converging, thence diverging and extending for about one fourth of scutal length. *Festoons* clearly marked, mostly slightly longer than wide. *Scutal surface* slightly convex. *Punctations* numerous, scattered over entire surface including festoons and lateral areas; shallow, coarse, non-discrete, large and medium size, mostly noncontiguous excepting some of those which run in shallow grooves centrally.

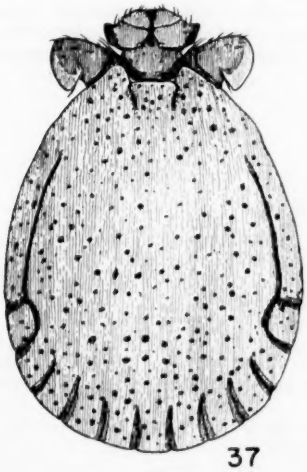
Legs: Coxae apparently unarmed but with faint spurs in the form of slight ridges; I basally bluntly rounded with a small point at basal angle; others subequal, rectangular, and with a very slightly raised ridge near the posterior, inner margin. *Trochanters* unarmed ventrally, I dorsally with huge shieldlike plate. *Tarsi* moderate, II to IV gradually tapering from about tarsal midlength to truncate apex; I very slightly humped, length and width about equal. *Pads* and *claws* moderate, pads reaching almost to apex of claws.

Ventral surface: Genital aperture between coxae II, with broadly rounded anterior margin

and rounded, subtriangular posterior margin. *Genital* and *anal grooves* as illustrated. *Spiracular plate* small, transversely oval with small, dorsally projecting tail.

FEMALE: This sex associated with males by similarity of most diagnostic details. *Size* slightly greater than that of male. *Body outline* of partially engorged specimen with sharply converging anterolateral margins, rounded lateral margins, and gradually rounded posterior margin.

Capitulum: Basis capituli three times as wide as long; lateral margins strongly diverging



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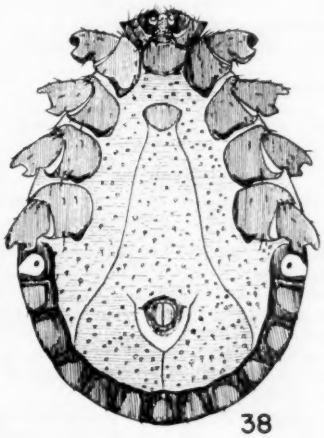

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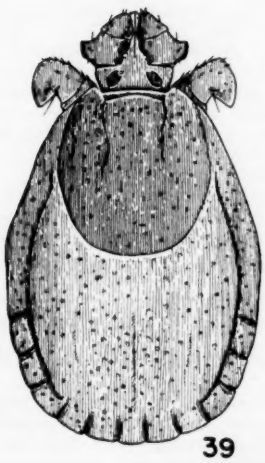

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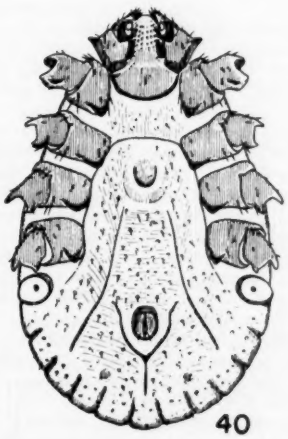

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Haemaphysalis cooleyi

MALE (Nuttall lot 3866): FIG. 37, dorsal view. FIG. 38, ventral view.
FEMALE (Allotype): FIG. 39, dorsal view. FIG. 40, ventral view.

anteriorly; posterior margin *very* slightly concave and with exceedingly small, rounded cornua; surface with a very few, shallow punctations, depressed centrally; porose areas small, indistinct, apparently oval, shallow. *Palpi* more elongate than those of male and with more strongly produced basal angle; apex narrower than in male; segment 3 four-fifths length of segment 2; seg-

ment 3 ventrally with broad, blunt, very short retrograde spur. *Hypostome* broadly elongate, denticles reaching from near base to corona (which is indefinite in the single mount); dentition 4/4 in files of seven to nine; denticles rather broad in outline.

Scutum measuring from 0.90 to 0.93 mm. long and from 0.76 mm. to 0.80 mm. wide (Nuttall's measurements of three specimens); *outline* diverging from scapulae almost to midlength, thence converging to moderately narrow but bluntly rounded posterior margin; *punctations* as in male but somewhat less dense; *cervical grooves* deep, almost straight and parallel, reaching about to midlength of scutum.

Legs. Coxae with outlines transversally rectangular; with inconspicuous, slightly raised ridges near inner posterior margins as in male. *Tarsi* and other segments, essentially as in male.

Ventral surface: Spiracular plate small, more rounded than that of male. Other features as illustrated.

NYMPH and LARVA: Unknown.

RELATED SPECIES: See below.

HAEMAPHYSALIS COOLEYI BEDFORD, 1929

(Figures 37 to 57)

Haemaphysalis cooleyi Bedford, 1929, pp. 494–495, Plate II on p. 498, male, female, and nymph briefly described and diagrammatically illustrated from three males, one female, and one nymph from Coombs' large-toothed hyrax, *Procavia capensis coombsi* Roberts, 1924, near Onderstepoort, Transvaal, 22 August 1928, R. A. Cooley and G. A. H. Bedford collectors; "also several engorged nymphs from (a springhaas) *Pedetes cafer* (syn. *caffer*) subsp. at Pienaars River, Transvaal, 1 October 1928, R. A. Cooley *legit*" (for comment on latter collection see second and third paragraphs below). Type specimens from *Procavia c. coombsi* in collections of Division of Veterinary Services, Onderstepoort; no material from *Pedetes* in this collection.

Bedford (1932, p. 289) mentioned original literature reference and collecting data.

Warburton (1933, p. 566–567) supplemented original description from three males (Nuttall lot 3866) sent by Bedford, taken from *Procavia capensis coombsi* near the original collecting locality on 5 June 1931; also stated that one male and one female sent by Cooley (tube 172) purporting to be this species and apparently being the so-called engorged nymphs from *Pedetes cafer* (referred to two paragraphs above) were actually specimens of *H. erinacei* Pavesi, 1884 (= *H. numidiana* Neumann, 1905).

Hoogstraal (1955, p. 230) footnote, indicated that after a long search he had found the material from *Pedetes* noted above (Cooley's tube number 172) in the Nuttall collection and that it represents an apparently undescribed species disinctly different from any known species. These specimens, preserved very soon after molting, appear to persons acquainted only with preserved ticks that they were engorged; it is my impression that they had not even fed but were taken crawling among the host's hair.

Material examined: Total: two nymphs, six males, five females.

Two nymphs, three males, and one female (including HOLOTYPE, ALLOTYPE, and PARATYPE nymph); Onderstepoort collection lot number 17A1 in two vials, one with type series and the other with two males and a nymph; data provided are same as that originally reported by Bedford except that an additional nymph is present, the date of collection is two days earlier, and only Bedford is listed as a collector.

Three males, Nuttall lot 3866 in British Museum (Natural History), material sent by Bedford to Warburton, mentioned under Warburton (1933) two paragraphs above (also tube 17*Z*; see two paragraphs above).

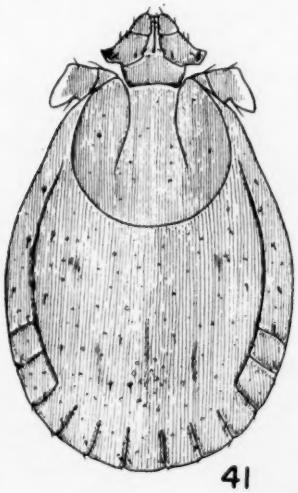
Four females from "dassie, Doornberg, New Bethesda, Cape Province, 18 December 1954", Onderstepoort collection Number 2974i.

REDESCRIPTION of *Holotype Male* (variation among other specimens included in parentheses): *Length* overall 1.9 mm., width 1.2 mm. (average). *Body* subcircular, slightly longer than wide; lateral margins convex medially, gradually converging anteriorly, posterior margin bluntly rounded (lateral margins may be parallel medially and more acutely converging anteriorly). Color reddish brown (straw color to dark brown).

Capitulum: Basis capituli almost twice as wide as long; lateral margins strongly diverging anteriorly but recurved at level of anterior sixth of length; cornua lacking but sometimes replaced by a slightly expanded rounded bulge; posterior margin very slightly convex; surface extremely faintly punctate. *Palpi* very short, medially most salient; 2.2 times as wide as long; basal margin dorsally reduced to a narrow, concave interval between insertion and acutely angled basolateral juncture; basolateral margin reaching almost to level of anterior margin of segment 2, thence acutely recurved and slightly (markedly) concave to base of segment 3; segment 3 (gradually

to) abruptly narrowing to broadly rounded apex (apex may be even more truncate); segment 3 about three-fifths as long as segment 2 (this proportion slightly variable); palpi dorsally with 12 hairs (apparently maximum number). *Palpi ventrally* with outline as from dorsal view except that the basal margin is even more reduced and the basolateral juncture is somewhat more rounded; segment 3 with a short, broad, bluntly rounded retrograde spur just barely overlapping anterior margin of segment 2. *Hypostome* stoutly elongate, bluntly rounded anteriorly, with weak but definite corona; dentition 4/4; denticles regular, overlapping, inner file denticles mostly smaller than those of outer files, in files of six to eight occupying the anterior three-fourths of the hypostome.

Scutum: Lateral grooves narrow, shallow but distinct, extending to anterior fourth of scutum; posteriorly enclosing first pair of festoons. *Cervical pits* short, fairly deep, (more or less) converging, *cervical grooves* absent (present on some specimens, shallow, extending to level of anterior margin of lateral grooves). *Festoons* (more or less) elongate; marked by shallow grooves; (usually) outlined by line or darker coloration. *Scutal* surface convex, smooth



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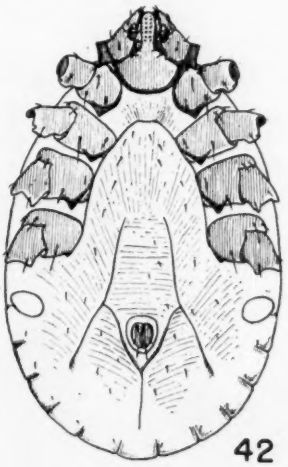

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Haemaphysalis cooleyi

NYMPH (paratype): FIG. 41, dorsal view. FIG. 42, ventral view.

(may be lightly rugose). *Punctations* few, widely and unevenly distributed over entire surface including festoons and lateral areas; consisting of about 50 (or fewer) medium to large, non-discrete, moderately deep punctations, and somewhat more numerous small punctations.

Legs: Coxae with weak, short, broad spurs (may be even more reduced or absent); coxa I bluntly or fairly sharply pointed basally (differ from side to side on same specimen); II to IV subrectangular, each with a ridgelike spur mildly pointed at inner posterior margin and just barely overlapping margin; coxal hairs few and short. *Trochanters* unarmed ventrally, I with large triangular shield dorsally. *Tarsi* short and slightly humped; fairly abruptly tapered from midlength to apex; I wider than long. *Pads* and *claws* moderate; pads not reaching base of distal curvature of claws.

Ventral surface: Genital aperture situated level with inner-anterior marginal junction of coxae II; broadly oval, with posterior margin more pointed than anterior margin. *Genital* and *anal grooves* distinct, as illustrated. *Spiracular plate* small, narrowly ovoid, broadly rounded ventrally and narrowed to a point dorsally.

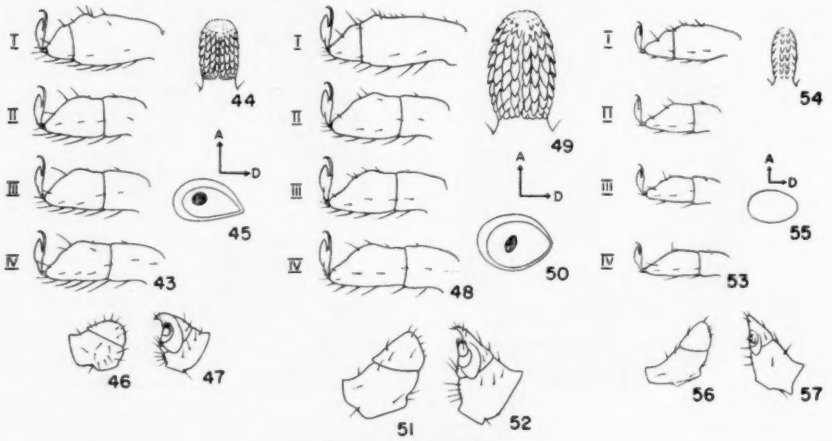
FEMALE: This sex is associated with males by similarity of diagnostic characters. *Size* varying from somewhat larger than that of male to as much as 2.50 mm. wide and 3.75 mm. long when engorged. *Body outline* slightly more linear than that of male; when engorged lateral margins are parallel.

Capitulum. Basis capituli three times as wide as long; lateral margins strongly diverging to anteriorly recurved portion as in male; posterior margin very slightly concave; posterolateral juncture pointed but cornua absent; surface impunctate; porose areas shallow, oval, tilted,

distant. *Palpi* more elongate than those of male but with similar outline except that from dorsal view basolateral margin is recurved at the level of midlength of segment *2* (not nearly at level of apex of segment *2*) ; segment *3* almost as long as segment *2*, more acutely triangular than in male; ventral retrograde spur of segment 3 essentially as in male. [Note that in the allotype (illustrated) palpi, both dorsally and ventrally, the emargination between the insertion and the basolateral juncture is much reduced. In the other specimens examined, this emargination is deeper, so that the basolateral juncture is more pointed]. *Hypostome* more elongate than that of male, bearing a small but distinct corona; dentition 4/4, denticles in files of seven or eight occupying the anterior four-fifths of the hypostomal length.

NYMPH: In all but one essential detail, the nymph shows close similarity of critical characters when compared with adults.

Capitulum. Palpi have a more elongate and triangular outline than those of the female but are otherwise similar except that (*notably*) the ventral basolateral juncture forms a small, distinctly produced spur. The ventral retrograde spur of segment 3 is small, moderately wide, bluntly rounded apically, and slightly overlaps the basal margin of segment 3. *Basis capituli* short and wide, 2.2 times as wide as long, cornua absent, basal margin concave, lateral margins diverging anteriorly. *Hypostome* with small denticles and corona, dentition *2/2*, seven denticles in inner file and eight in outer file.



Haemaphysalis cooleyi

MALE: FIG. 43, tarsi I to IV, lateral view. FIG. 44, hypostome, ventral view. FIG. 45, spiracular plate. FIG. 46, palpus, dorsal view. FIG. 47, palpus, ventral view.

FEMALE: FIG. 48, tarsi I to IV, lateral view. FIG. 49, hypostome, ventral view. FIG. 50, spiracular plate. FIG. 51, palpus, dorsal view. FIG. 52, palpus, ventral view.

NYMPH: FIG. 53, tarsi I to IV, lateral view. FIG. 54, hypostome, ventral view. FIG. 55, spiracular plate. FIG. 56, palpus, dorsal view. FIG. 57, palpus, ventral view.

Scutum: Outline subcircular, length and width almost equal, margins rounded. *Cervical grooves* slightly converging for one-third their length, thence gradually diverging to posterior third of scutal length. *Punctations* rare, small, shallow.

Legs: Coxae essentially as in female except for their slightly narrower outline; spurs slightly more pointed and slightly more removed from inner margin. *Trochanter* I with fairly large, pointed shield dorsally. *Tarsi* similar to those of adult but not quite so robust. *Pads* and *claws* similar to those of adults.

Spiracular plate very small, ovoid, lacking dorsal projection.

LARVA: Unknown.

RELATED SPECIES

Members of the "*H. orientalis* group" are related to the "*H. hoodi* group" of Africa, Madagascar and Asia, and are separated from all forms of the "*H. leachii* group" by the presence on the ventral surface of palpal segment 3 of a short, wide,

bluntly rounded, retrograde spur which slightly if at all overlaps the basal margin of segment 3. The comparatively long and narrow ventral retrograde spur of segment 3 in the "*H. leachii* group" is a remarkably constant feature in this otherwise extremely variable complex of Africa and Asia.

H. bequaerti sp. nov. superficially resembles *H. leachii muhsami* Santos Dias 1954 (comb. nov.). [This latter tick, which Santos Dias (1954B) described as a full species, has previously been referred to by workers on African ticks as "the small form of *H. leachii*" or (Theiler, 1943) as "temporarily *H. leachi* var. *indica*". Numerous intergrades between the subspecies *muhsami* and the nominal subspecies of *H. leachii* exist in nature]. *H. bequaerti* sp. nov. can be easily differentiated from *H. leachii muhsami,* and even more easily from *H. l. leachii,* by the shape of the ventral spur of palpal segment 3, as noted above; greater lateral concavity of palpi, and reduction of basal palpal spurs; smaller spiracular plate with narrower and more elongate dorsal projection; typical enclosure of two pairs of festoons by lateral grooves (usually only one pair enclosed in *H. leachii* subsp.); coxae without spurs and with fewer and much shorter hairs and I bluntly rounded basally (coxae with overlapping, definite spurs on at least two pairs, with about ten hairs some of which are almost as long as the coxae, and with I pointed basally in *H. leachii* subspp.). The palpal characters listed above, differences in scutal outline and punctation, and absence of overlapping coxal spurs easily separate the female of the new species from that of *H. leachii* subspp. Most of these differentiating characters also apply to the two other members of the "*H. orientalis* group", thereby separating them from *H. leachii* subspp.

Nymphs of *H. bequaerti* sp. nov. and of *H. cooleyi* have a pronounced ventral basolateral spur of the palpi, similar to that of *H. leachii.* In adults of the first two species, these spurs are considerably reduced or lacking. In these nymphs, too, the ventral retrograde spur of palpal segment 3 tends to conform to the nymphal *H. leachii* type.

Several or all of the following characters are among those which separate adults of the hyrax parasites from those of *H. hoodi* subspp.: diverging basis capituli, angled basal margin of palpi, essentially unarmed coxae, smaller spiracular plates, enclosure of second festoon (only in *H. bequaerti* sp. nov.), absence of tarsal hooks, longer tarsal pads, etc. Related species and extracontinental subspecies of *H. hoodi* also differ markedly.

Members of the "*H. orientalis* group" are removed from *H. erinacei* Pavesi, 1884 (syn. *H. numidiana* Neumann, 1905) (cf. Hoogstraal, 1955), with which Santos Dias (1954A) compared *H. orientalis* (= *H. zambeziae*), by a number of obvious differences. *H. erinacei* in both sexes has distinct, overlapping coxal spurs and long hairs, and has a narrowly elongate ventral retrograde spur on the third palpal segment (more like *H. leachii* subspp. in these respects); the male has a rectangular basis capituli, fairly large cornua, etc. Actually, *H. erinacei* may be considered as a part of the *H. hoodi* complex.

Within the "*H. orientalis* group", males of *H. bequaerti* sp. nov. differ from the others as follows: cornua present (almost obsolete in *H. orientalis,* obsolete in *H. cooleyi*), palpi with more acutely pointed, spurlike basolateral juncture than in other species, scutal punctations moderately numerous (numerous in *H. orientalis,* few in *H. cooleyi*), lateral grooves typically enclosing two pairs of festoons (enclosing one

pair in both other species), coxae without overlapping spurs (very small spurs in *H. cooleyi*), moderate dorsal shield on trochanter I (large shield in other species), etc. The females are as easily separated. The female scutum in *H. bequaerti* sp. nov. is narrowly rounded posteriorly and bears a moderate number of punctations while those of the other two species are broadly rounded posteriorly and have more or fewer punctations. *H. bequaerti* sp. nov. females typically bear cornua on the basis capituli (sometimes much reduced), which are absent in the other species. Only females of *H. cooleyi* have coxae with overlapping spurs.

DISCUSSION

Hosts and host-parasite relationships. The data, and their implications, concerning hosts and host-parasite relationships of the *"H. orientalis* group", in comparison with those of related groups, are of interest. Contemporary studies indicate a fairly strict host-parasite specificity in most haemaphysalid groups. The *"H. orientalis* group" feeds on hyraxes and no other animals are known to serve as hosts.

The host indicated by Santos Dias (1954A) for *"H. zambeziae",* a synonym of *H. orientalis,* was a klipspringer antelope, *Oreotragus oreotragus* subsp. However, Theiler (1955) has quoted the following passage from a letter of Santos Dias: *"R(hipicephalus) serranoi* and *H. zambeziae:* Chequi a uma conclusão interressante acerca destas especies. Ambas tem como principais hospedeiros os hiraxes. Em ambas o conscutum e densamente pontudao, parecendo que do seu habitat terio resultade como que un adjustamento de caracteres". It appears obvious, therefore, that the designation of an antelope as the host of *"H. zambeziae",* in the original description, was in error.

Among the hyraxes (HYRACOIDEA, PROCAVIIDAE), this group of ticks appears from present evidence, to feed indiscriminately on either of both genera of rock hyraxes, *Heterohyrax* and *Procavia,* (locally called "dassies" or "coneys"), but we do not know that it feeds on the arboreal hyraxes, *Dendrohyrax.* The usage of caves by the first two genera, as compared with the arboreal habitats of the latter, suggests that the hosts' living habits may favor parasitism by these ticks.

Furthermore, haemaphysalid-host relations indicate that these ticks must be among the oldest of the ixodids, except possibly for the highly modified genus *Ixodes.* The phylogenetic position and great age of hyraxes among the more spectacular and much more recent bulk of the African mammal fauna is, therefore of considerable interest.

"Hyraxes are certainly old Africans. They are first known in the early Oligocene of Egypt, and probably arose in Africa in the Eocene, or perhaps Paleocene. They seem never to have spread far outside Africa. Only an early Pliocene occurrence in Greece is outside their present range. Some other African mammals may be nearly or quite as old in the continent (among insectivores, primates, proboscideans, for example). The conspicuous ungulates are probably relatively late in Africa and in any case have closer affinities (than have the hyraxes) with former and present Eurasian forms. The last remark applies also to some of the insectivores and also the primates and proboscideans. The hyraxes, in short, are unusual among recent African mammals in being both old there and almost confined to the continent throughout their history". [Simpson, 1955].

The host-specificity of various groups of haemaphysalid ticks makes the investi-

gation of the actual status of reports concerning haemaphysalids on hyraxes of more than ordinary interest.

Nuttall and Warburton (1915, p. 469) stated that Nuttall lot 1223, consists of males and females of *H. leachii* from *Procavia brucei* and *P. abyssinica,* collected in May, 1901, at Harrar, Ethiopia by Dr. E. Brumpt, and that these two lots were small specimens; in the same sentence they also indicated specimens from a jackal and a hyena. Nuttall lot 1223 now consists of two males and female labelled as from *Helogale parvula,* a type of mongoose. These three specimens are small but typical *H. leachii.* However, the label also bears the letter "D". This may mean either that the hosts were mixed and those from hyraxes were separated into a different vial with another letter, which cannot now be found, or possibly that the designation of hyraxes as hosts for lot 1223 was later found to be incorrect. Also, on page 471, Nuttall and Warburton indicated that they had specimens of *H. leachii* from *Hyrax* sp. at Monkey Bay, Nyasaland, 23 May 1913, Dr. W. C. Wigan *legit* (Nuttall lot 2366). It would be of interest to restudy these specimens, but they cannot now be found in British Museum (Natural History) collections.

That the comparatively versatile *H. leachii* on occasion does attack hyraxes is attested by a few records sent by Dr. Theiler from the Onderstepoort collection. These include three lots of *H. l. leachii* from South African *Procavia capensis,* and two lots of *H. leachii muhsami,* one from *Heterohyrax b. brucei* in Uganda and one from *Procavia habessinica jacksoni* (= *P. mackinderi zelotes*) in Kenya.

While this group of three hyrax-parasitizing species is related to the bird-parasitizing *H. hoodi* complex of southern Asia, Madagascar, and Africa, it also displays relationships with the *H. leachii* group, from which the *H. hoodi* complex or group is also derived. Thus, *H. bequaerti* sp. nov. is, within the "*H. orientalis* group", more like *H. leachii indica* of India and *H. leachii muhsami* of Africa than are *H. orientalis* and *H. cooleyi. H. bequaerti* sp. nov. of northeastern tropical Africa is geographically closest to the apparent source of this group, India or Malaya. Further south in eastern Africa, *H. orientalis* has become increasingly more differentiated from the basic *H. leachii* facies. And still further south, in South Africa, *H. cooleyi* has evolved as a related yet exceedingly distinct member of the group. It is therefore, tentatively suggested that *H. bequaerti* sp. nov. is the prototype from which the other two members of the "*H. orientalis* group" have evolved.

On continental Africa, some haemaphysalids feed on lower vertebrates (e.g. *H. hoodi* on birds), others attack the oldest or the most primitive of the mammals (e.g. the "*H. orientalis* group" of hyraxes and *H. erinacei* of hedgehogs), while yet others have adapted to more recent mammals (e.g. *H. houyi* and *H. calcarata* on ground squirrels and *H. aciculifer* and *H. parmata* on antelopes). The highly variable *H. leachii* of Africa retains the typical *leachii* group predilection for carnivores as hosts of the adult stage, a character also true of many southern Asiatic species of the group. In Africa, the immature stages of *H. leachii* feed on burrowing rodents (no biological studies have been reported from Asia), and the carnivore-infesting adults only rarely attack other animals such as antelopes and cattle. Curiously, a fairly distinct form of *H. leachii* appears to thrive in all its stages on African mole-rats (RODENTIA, RHIZOMYIDAE; *Tachyoryctes*).

Distribution. The contemporary range of hyraxes includes much of tropical and southern Africa and extends into southeastern Egypt, Sinai, Palestine, Syria,

and Arabia, besides Libya and Algeria. Outside of this area, fossil remains, as stated above, are known only from Greece.

Within the hyraxes' range, we have records of haemaphysalid parasites only from eastern and southern Africa. They are absent in many areas where hyraxes occur, and, when present, appear to occur only in small numbers. I have examined without success many hyraxes for ticks in Arabia, Sinai, southeastern Egypt, Kenya, and several areas of the Sudan.

H. bequaerti sp. nov. is known from Equatoria Province of the Sudan, both west of the Nile near the borders of French Equatorial Africa and the Belgian Congo, and east of the Nile; also from Karamoja District of Uganda and from Naishai in Kenya. *H. orientalis* inhabits Nyasaland and the Zambesi area of Mozambique. *H. cooleyi,* the South African representative of this group, is known from Transvaal and Cape Province.

Associated tick species: Few other ticks are known to attack hyraxes. In Uganda, Dr. Haddow took three immature *Rhipicephalus* sp. with his series of *H. bequaerti* sp. nov. In the Sudan, nymphal *R. mühlensi* Zumpt, 1943, have been found together with the same haemaphysalid as well as larvae of *Argas brumpti* Neumann, 1907, and a few *Rhipicephalus s. sanguineus* (Hoogstraal, 1954A). In the Cameroons, outside of the known range of these haemaphysalid ticks, adults of *R. ziemanni* Zumpt, 1943, infest hyraxes (Hoogstraal 1954B). In Southeastern Egypt and in the Yemen (Arabia), I have found only a few larval *Hyalomma* ticks on hyraxes. Neumann (1911), Schwetz (1927), and Bedford (1932) recorded specimens of *Ixodes rasus* from rock hyraxes from central to southern Africa.

All of these appear to be incidental records except for those of *Argas brumpti,* which is a fairly common parasite of hyraxes though it also attacks mammals and reptiles inhabiting rock niches, caves, dens, and dry burrows (Hoogstraal, in press). *Rhipicephalus distinctus* Bedford, 1932 (syn. *R. punctatus* Bedford, 1929) of South and Southwest Africa originally reported from rock hyraxes, parasitizes other animals only exceptionally. Whether specimens of *R. serranoi* Santos Dias, 1950, originate from klipspringer antelopes or from hyraxes is impossible to determine.

The only records of which I am aware for parasitism of tree hyraxes, *Dendrohyrax,* by ticks are those of Bequaert (1931) and Schoenaers (1951) for *Ixodes rasus* in the Belgian Congo.

The few available records of *H. leachii* subspp. attacking hyraxes have been presented under *Hosts and Host-parasite Relationships* above.

Potential disease relations. Several of the Sudan specimens of *H. bequaerti* sp. nov. were obtained from the same hyrax hosts in which a new genus and species of piroplasm, *Echinozoon hoogstraali* Garnham, 1951, was found (Garnham, 1951). [The earlier host identification has been changed by Setzer (in press)]. It is quite possible that these ticks may be the vector of this strange organism.

ACKNOWLEDGEMENTS

I am most grateful to Dr. G. Theiler of Onderstepoort for the privilege of examining the type specimens and additional series of *H. cooleyi* and Uganda specimens of *H. bequaerti* sp. nov.; to Dr. G. O. Evans and Mr. E. Browning of British Museum (Natural History) for the loan of specimens of *H. orientalis* and of *H. bequaerti* sp. nov.; to Mr. R. Alison for assistance in collecting the Lui paratypes of

H. bequaerti sp. nov.; to HMC D. K. Lawless, USN, for assistance in collecting the Sudan hosts of *H. bequaerti* sp. nov., and to Mr. Glen M. Kohls for reviewing the manuscript.

The new species described herein is named for Dr. J. Bequaert, Museum of Comparative Zoölogy, Harvard University, in recognition of his outstanding contributions to our knowledge of parasitic arthropods of Africa and elsewhere, and of his assistance and many kindnesses shown me when I was commencing the study of ticks.

SUMMARY

Haemaphysalis bequaerti sp. nov. is described from adults, nymphs, and a larva from the Sudan, Uganda, and Kenya. *H. orientalis* Nuttall and Warburton, 1915 (comb. nov.) of Nyasaland and Mozambique, is redescribed, as is *H. cooleyi* Bedford, 1929, of South Africa. *H. zambeziae* Santos Dias, 1954, is a synonym of *H. orientalis*. These three species are referred to as the "*H. orientalis* group."

The host predilection of these species for hyraxes is discussed in relation to the great age of these mammals in the African fauna and the habits of related species and groups (*H. leachii, H. hoodi,* and *H. erinacei*). Hyraxes are apparently rarely attacked by other species of ticks; available records are provided. The possible vector relation of *H. bequaerti* sp. nov. to a strange piroplasm of hyraxes is noted.

H. muhsami Santos Dias, 1954, is herein considered as a subspecies of *H. leachii* (Audouin, 1827) (comb. nov.). This is the form previously referred to by workers on African ticks as the "small form of *H. leachii*," as *H. leachii indica,* or as *H. leachii* near *indica.*

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RESEARCH NOTES

A CARPET BEETLE LARVA (COLEOPTERA : DERMESTIDAE) FROM THE DIGESTIVE TRACT OF A WOMAN

On July 21, 1955 a woman forty-three years old in London, Ontario experienced a sensation of peri-anal itching, and suspecting the presence of pin-worms, examined a stool following a bowel movement. In it was found a small, dead "worm". When examined by the writer this proved to be a larva, 7 mm. long, of a beetle of the genus *Attagenus* as identified with keys in B. E. Rees (1943. Classification of the Dermestidae (larder, hide, and carpet beetles) based on larval characters, with a key to the North American genera. U.S. Dept. Agric., Miscell. Publ. No. **511**). It closely resembled the larva of *Attagenus piceus* figured by A. Gibson and C. R. Twinn (1939. Household insects and their control. Dept. Agric., Canada, Publ. No. **642**). *A. piceus* is a beetle of cosmopolitan distribution and its larva feeds on furs, skins, woolen fabrics, stored grains and cereals (Busvine, J. R. 1951. Insects and Hygiene. Methuen and Co., London).

R. Matheson (1950. Medical Entomology. Comstock Publ. Co., Ithaca) points out that cases of canthariasis, the infection of tissues by beetle larvae, relate to larvae of the Dermestidae and Tenebrionidae and that infection probably occurs through eating of food containing eggs or larvae. H. Liggett (1931. J. Amer. Med. Assn. **96**: 1571–1572) reports a case of larvae of *A. piceus* infecting the nose of a girl. In the case of the woman at London, as reported by the present writer, the larva was probably ingested with food.

Clinical data in this case were contributed by C. A. Cline, M.D., who was the physician investigating the case and the larva was submitted to the writer, for identification, by W. M. Wilson, M.D., of the Regional Laboratory, Ontario Department of Health, London.—W. W. JUDD, *Department of Zoology, University of Western Ontario, London, Ontario.*

PARASITIC COPEPODS (ARGULIDAE) ON AMPHIBIANS

The parasitic copepods of the family Argulidae, commonly known as fish lice, are known to infest many fishes. (See Meehean, O. L., Proc. U. S. Nat. Mus. Vol. 88, 1940, and Wilson, C. B., Proc. U. S. N. M., Vol. 94, 1944). We wish here to report instances of parasitism on a salamander and a frog tadpole by a species of these copepods.

The first case involved a specimen of the perennibranch salamander, *Pseudobranchus s. axanthus*, collected near Gainesville, Florida. About December 1, 1939, a lump was noted on the head of an adult that had been in an aquarium in the laboratory for several weeks, but the lump was mistaken for a mass of detritus. However, the salamander gradually became weak and inactive, and on December 7, it was found lying on its side on the bottom of the aquarium. The lump was still present, but now was about half an inch behind the head. Upon close examination, it was found that what had been mistaken for detritus was in reality a parasitic copepod *Argulus americanus* (Wilson). The copepod was removed and the *Pseudobranchus* was put back in the aquarium with fresh water, but it continued to weaken and died on December 9.

The second instance concerned a tadpole of the frog, *Rana heckscheri* Wright. While large tadpoles of this species were being collected during November, 1955, at Newman's Lake, about four miles east of Gainesville, a specimen was found that had two individuals of *A. americanus* attached to it. Since the tadpole as well as the copepods were preserved shortly after capture, we cannot report on how the parasites affected the well-being of the tadpole.

Although the fact that these parasites make use of aquatic amphibians as hosts is not unexpected, it seems to be unrecorded. We wish to thank Dr. Horton H. Hobbs for identifying the copepods for us.—COLEMAN J. GOIN AND LARRY H. OGREN, *Department of Biology, University of Florida, Gainesville.*

SHUNSENNIA BIPLUMULOSA, A NEW SPECIES OF CHIGGER FROM JAPAN (ACARINA: TROMBICULIDAE)*

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Incidental to a study of Japanese ectoparasites of several hundred host animals, many Acarina were collected.

Four specimens of a trombiculid mite from a Japanese shrew-mole are the basis for the new species described in this paper.

Shunsennia biplumulosa, n. sp.

Body: Body size derived from measurements in microns of four partially engorged specimens. Length, 400 to 562, average 497; width, 237 to 326, average 300. Color, pale orange. Double eyes present, though small, inconspicuous and well removed from the lateral margin of the scutum. Spiracles and tracheae absent. Anal opening slightly anterior to midpoint from coxa III to posterior margin, probably varying with the degree of engorgement.

Gnathosoma: Cheliceral base and palpal femur punctate. Cheliceral blade with five minute ventral teeth and a tricuspid cap. Palpal femoral and genual setae with rather long side branches. Lateral palpal tibial seta simple. Dorsal and ventral palpal tibial setae with short barbs. Palpal claw apparently two pronged. Palpal tarsus with six feathered setae and a short spine. Galeal seta simple.

Legs: All legs with six segments ending with two claws and a claw-like empodium. Coxal setae 2-2-2 (one specimen had three setae on one coxa I). With the following specialized setae: Leg I, 2 genualae, 1 microgenuala, 2 tibialae, 1 microtibiala, 1 spur, 1 subterminala, 1 pretarsala; leg II, 2 genualae, 1 microgenuala, 2 tibialae, 1 microtibiala, 1 spur, 1 pretarsala; leg III, 1 genuala, 1 tibiala. The description of *S. tarsalis,* J. and T., the genotype species, mentioned the "considerable variation in degree of feathering on the leg setae." The same condition was noted on the leg setae of *S. biplumulosa.*

Scutum: Broadly rectangular, about three times as wide as long; anterior margin slightly convex mesally and posterior margin with a definite concavity. Scutal setae similar in diameter and quality. Sensillae simple. Scutal measurements (mean of four specimens): AW-99, PW-127, SB-41, ASB-47, PSB-22, AP-42, AM-86, AL-71, PL-93, SD-69, S-95.

Setae: Approximately 60 to 63 dorsal setae and 68 to 77 ventral setae, irregular in arrangement and variable in form from plumose to barbate. The dorsal setae varied in length from 80 to 105, while the ventral setae varied from 32 to 88, the shorter, anterior setae being more plumose than the longer, posterior setae. There seemed to be no definite humeral setae. A single pair of sternal setae was located between coxae II and III.

Type data: Holotype, taken from a shrew-mole, *Urotrichus talpoides,* Mt. Fuji, Yamanashi Prefecture, Japan, August 17, 1954; deposited in the United States National Museum, No. 2222. One paratype, same data as the holotype, deposited at the Medical Zoological Laboratory, Institute for Infectious Diseases, University of Tokyo. One paratype, same data as the holotype, deposited at the Rocky Mountain Laboratory, Hamilton, Montana. One paratype, same data as the holotype, retained by the author.

All collections were made by personnel of the Preventive Medicine Unit, Third Marine Division.

DISCUSSION

Shunsennia biplumulosa is a chigger possessing morphological similarities to both *Chatia* and *Shunsennia,* emphasizing the close relationship of these two genera. Brennan (1946, 1955) stated that in the monotypic genus *Chatia* the empodium is lacking on all tarsi, certainly a distinctive taxonomic character. *S. biplumulosa*

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* The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

possesses empodia on all tarsi, but otherwise agrees with the present generic description for *Chatia*. *S. biplumulosa* also agrees with the description for *Shunsennia* as given by Jameson and Toshioka (1953), in all but the presence of cheliceral teeth. Brennan (1955) on finding cheliceral teeth on *Chatia*, reexamined the types of *Shunsennia tarsalis* and *S. ochotona*, but found they lacked these teeth. The minute cheliceral teeth on *S. biplumulosa* are not discernible except in full lateral view, and are not visible on two of the four type specimens. I am indebted to Dr. Brennan for bringing this character to my attention when he examined the specimen on deposit with the Rocky Mountain Laboratory. The presence or absence of cheliceral teeth is of taxonomic value, but when the teeth are so minute as to require a certain position of the specimen under oil immersion, the generic value is decreased in favor of more easily recognized differences. Until more information becomes available, it seems desirable to place *biplumulosa* with the genus *Shunsennia*, leaving *Chatia* a monotypic genus. In doing so, the generic description for *Shunsennia* should be amended to include the presence or absence of ventral cheliceral teeth, and a palpal claw with two to four prongs.

S. biplumulosa differs from *tarsalis* and *ochotona* in having a single pair of sternal setae, two setae on each coxa (the basis for the specific name), simple galeal seta and generally fewer setae on dorsum and venter.

The area where the host was trapped was at an altitude of 4000 to 4500 feet in a wild, virgin forest on the northwestern slope of Mt. Fuji. Of nine *Urotrichus* trapped in that area, only one was found to harbor any chiggers.

Key to species of SHUNSENNIA

1. One pair of sternal setae; two setae on each coxa; minute teeth on ventral surface of cheliceral blade ..... *biplumosa*, n. sp.
   Two or three pairs of sternal setae; one seta on coxa II; no teeth on chelicerae 2
2. Three pairs of sternal setae; usually three setae on coxa III; sensilae barbed basally; palpal claw with four prongs ..... *ochotona* (Radford, 1942)
   Two pairs of sternal setae; one seta on coxa III; sensillae without basal barbs .... *tarsalis* Jameson & Toshioka (1953)

The writer wishes to acknowledge the encouragement of Dr. J. M. Amberson, CAPT, MC, USN, for his interest in the Japanese ectoparasite survey, and the helpful advice of Dr. G. W. Wharton.

SUMMARY

Shunsennia biplumosa, a new species of larval chigger mite, is described from a Japanese shrew-mole, and a key to three species is provided.

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PLATE I

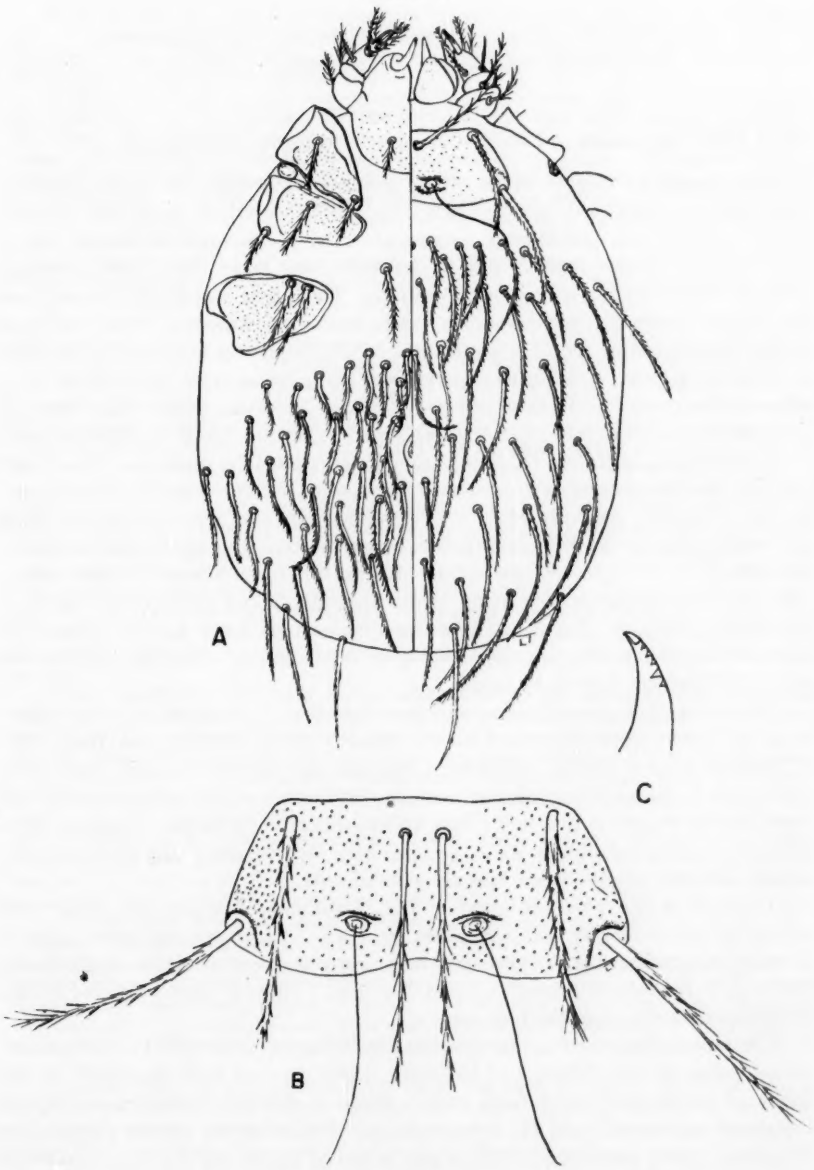

PLATE I

Diagnostic structures of *Shunsennia biplumosa*

FIG. A. Dorso- ventral aspect of larva.
FIG. B. Scutum.
FIG. C. Ventral teeth on cheliceral blade.

A NEW SPECIES OF CHIGGER MITE, HANNEMANIA HEGENERI (ACARINA; TROMBICULIDAE)*†

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Two species of chigger mites of the genus *Hannemania, H. dunni* Sambon, 1928, and *H. penetrans* Ewing, 1931, have been described from the Eastern United States. *H. dunni* was recovered from *Desmognathus fuscus fuscus* from "Eastern United States" and *H. penetrans* was taken from *Rana clamitans* collected at Great Falls of Potomac, Virginia. Two other species are known from the United States, *H. eltoni* Sambon, 1928, from San Antonio, Texas, on *Rana pipiens sphenocephala* and *H. hylae* (Ewing, 1925) from San Diego Co., California, on *Hyla arenicolor.* During the years 1949–1952, larvae were taken from *R. p. sphenocephala* collected in the vicinity of Sarasota, Florida, which differ from all of the above species as far as can be ascertained. They are herein described as new.

The status of *H. dunni, H. eltoni,* and *H. penetrans* is in question. The disposition of the type specimens of Sambon's two species, *H. dunni* and *H. eltoni* is not known. The host specimen from which Sambon obtained specimens of *H. eltoni* has been located in the British Museum, and some topotypic specimens have been obtained by H. S. Fuller of the Army Medical Graduate School. Unfortunately some of these specimens lack many of the appendages and consequently many of the diagnostic setae. These and other specimens from *Rana pipiens* collected at Camp Bullis, 17 miles from the type locality of San Antonio, Texas, by R. B. Loomis and J. S. Wiseman, have been studied.

The status of *H. dunni* is more uncertain than that of *H. eltoni.* No type specimens are known to be extant and the only locality given in the original description is "Eastern United States." However, the host was identified as the dusky salamander, *D. f. fuscus,* and on the basis of the distribution of this subspecies it would seem that the chiggers (and host) were collected north of Florida. Chiggers taken from *D. f. fuscus* throughout its range have been studied and found not to be conspecific with the proposed new species from Florida.

The type of *H. penetrans* is extant (U. S. National Museum No. 1028), but since it is from a locality within the area from which *H. dunni* was taken, there is a strong suggestion that *H. penetrans* may in reality be a synonym of *H. dunni.* The type of *H. hylae* is extant (USNM No. 956). Both *H. penetrans* and *H. hylae* differ markedly from the new species.

The terminology used is that proposed by Wharton *et al* (1951). A pointed, striated sensory seta (Type 2 of Wharton, 1948) has not been described for the femur of the larva of trombiculid mites. Setae of this description have been encountered on femora I and III in this species. Following the system proposed by Wharton (1948) whereby this type of seta is named for the leg segment from which it originates, it is termed the "femorala."

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Hannemania hegeneri n. sp.

Body: Length and width of laboratory-reared, unengorged holotype, 278 by 165 microns (engorged specimens average 800 by 500 microns). Color in life orange-red. Striae not prominent. Eyes 2/2 on an ocular plate, approximately equal in size. Anus located at about the level of the third row of ventral setae.

Gnathosoma: Cheliceral bases not punctate. Blade of chelicera expanded distally; laterally armed with a row of approximately 18 backwardly directed teeth which increase progressively in size from tip; shank with hook near level of cheliceral blade. Palpal setae: coxal, femoral, genual, and ventral and dorsal tibial setae branched; lateral tibial nude. Palpal claw trifurcate, the median tine longer and stouter. Palpal tarsus one and one-half times as long as wide, with 5 branched setae, and a spur. Galeal seta minutely branched, filamentous.

Scutum: Roughly heart-shaped, with anteromedian projection; strongly convex posteriorly; without projection, scutum is nearly as broad as long. Puncta scattered over entire scutum but more concentrated in area of sensillary bases. Sensillary bases posterior to posterolateral setae, and separated by twice the diameter of a base. Sensillae long, filamentous, without branches. Setae barbed. Anteromedian projection 17 microns long.

Scutal data (in microns) for holotype follow, and in parentheses the minimum, maximum, and mean measurements of the paratypes in that sequence: AW–41 (39–49/44); PW–57 (53–61/58); SB–21 (19–23/22); ASB–53 (46–60/53); PSB–23 (22–32/25); AP–12 (12–20/16); AM–25 (21–30/25); AL–31 (25–35/31); PL–53 (47–57/53); S–75 (66–77/many broken).

Legs: All legs with 6 segments. Setal distribution as follows: Leg I: Coxa with 2 branched setae; trochanter with one branched seta; femur with 6 branched setae and 1 nude seta herein termed the femorala; genu with 4 branched setae, 12 genualae (paratypes vary from 8 to 13) and 1 microgenuala; tibia with 8 branched setae, 2 tibialae, and 1 microtibiala; tarsus with about 25 branched setae, 1 spur, 1 microspur, 1 subterminala, 1 parasubterminala, and a pretarsala. Leg II: Coxa and trochanter with 1 branched seta each; femur with 5 branched setae, no femoralae; genu with 4 branched setae, 4 genualae, and 1 microgenuala; tibia with 6 branched setae, 2 tibialae, and a microtibiala; tarsus with about 18 branched setae, 1 spur, a microspur, and a pretarsala. Leg III: Coxa and trochanter each with 1 branched seta; femur with 4 branched setae and 5 nude setae (paratypes possess from 4 to 7 femoralae with an average of 5); genu with 4 branched setae and 9 genualae (paratypes vary from 7 to 9); tibia with 6 branched setae and a tibiala; tarsus with about 17 branched setae, no nude setae. All tarsi are terminated by a pair of sickle-shaped claws and a longer, more slender, claw-like empodium between.

Setae: Dorsal setae about 58 in number coarsely barbed and similar in form to the scutal setae. Arranged in somewhat irregular rows, but approximately according to the pattern 2-4-10-8-14-12-6. One pair of humeral setae, similar to general dorsal setae in shape but slightly longer. Ventral setae: anterior sternals wanting; posterior sternals present, similar in form but about twice as long as other ventral setae; ventral setae approximately 50 in number.

Materials: Holotype and six paratypes are unengorged laboratory-reared larvae, which were reared from specimens recovered from the southern leopard frog, *Rana pipiens sphenocephala* collected in the environs of Sarasota, Florida. Eight additional paratypes are engorged larvae collected from the same host species and locality. Holotype and one engorged paratype have been deposited in the U. S. National Museum (USNM No. 2208). One engorged and one unengorged paratype will be placed in each of the following depositories: British Museum (Natural History), Rocky Mountain Laboratory, South Australian Museum, University of Maryland, University of Kansas, and the author's collection.

Diagnosis: Hannemania hegeneri possesses nude setae, or femoralae, on femora I and III, and can be separated from all other nearctic species on this basis except *H. eltoni,* the species to which it is most closely related. Four or more femoralae are possessed by *H. hegeneri* on leg III, while *H. eltoni* possesses fewer than this number. Also scutal setae PL and AM are longer on *H. hegeneri* than on *H. eltoni.* Both species possess femoralae on leg I as well as on leg III.

Remarks: That this species might be the species described by Sambon (1928) as *H. dunni,* is not likely. The distribution of the type host of *H. dunni, Desmognathus fuscus fuscus,* does not coincide with the known distribution of *H. hegeneri. Desmognathus fuscus,* in any of its subspecies, is not known to occur in the environs of Sarasota, Florida.

This species has been named for Mr. Robert Hegener of the Hegener Research Supply, Sarasota, Florida, who has been collecting these mites for many years, and from whom the material for this investigation was obtained.

Hannemania hegeneri has been reared through its complete life cycle in the laboratory using eggs of the collembolan, *Sinella curviseta* Brook, as the post-larval

food (Hyland, 1950; Lipovsky, 1951), and various species of both anurans and urodeles as hosts for the larvae.

SUMMARY

A new trombiculid mite, *Hannemania hegeneri*, is described from the frog, *Rana pipiens sphenocephala*, collected in the vicinity of Sarasota, Florida. It has been reared through its life cycle in the laboratory.

ACKNOWLEDGMENT

Thanks are extended to H. S. Fuller and to R. B. Loomis for the loan of specimens, to E. W. Baker of the U. S. National Museum for making specimens and facilities available for study, and to G. W. Wharton of the University of Maryland for advice and criticism.

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EXPLANATION OF PLATE

Hannemania hegeneri new species

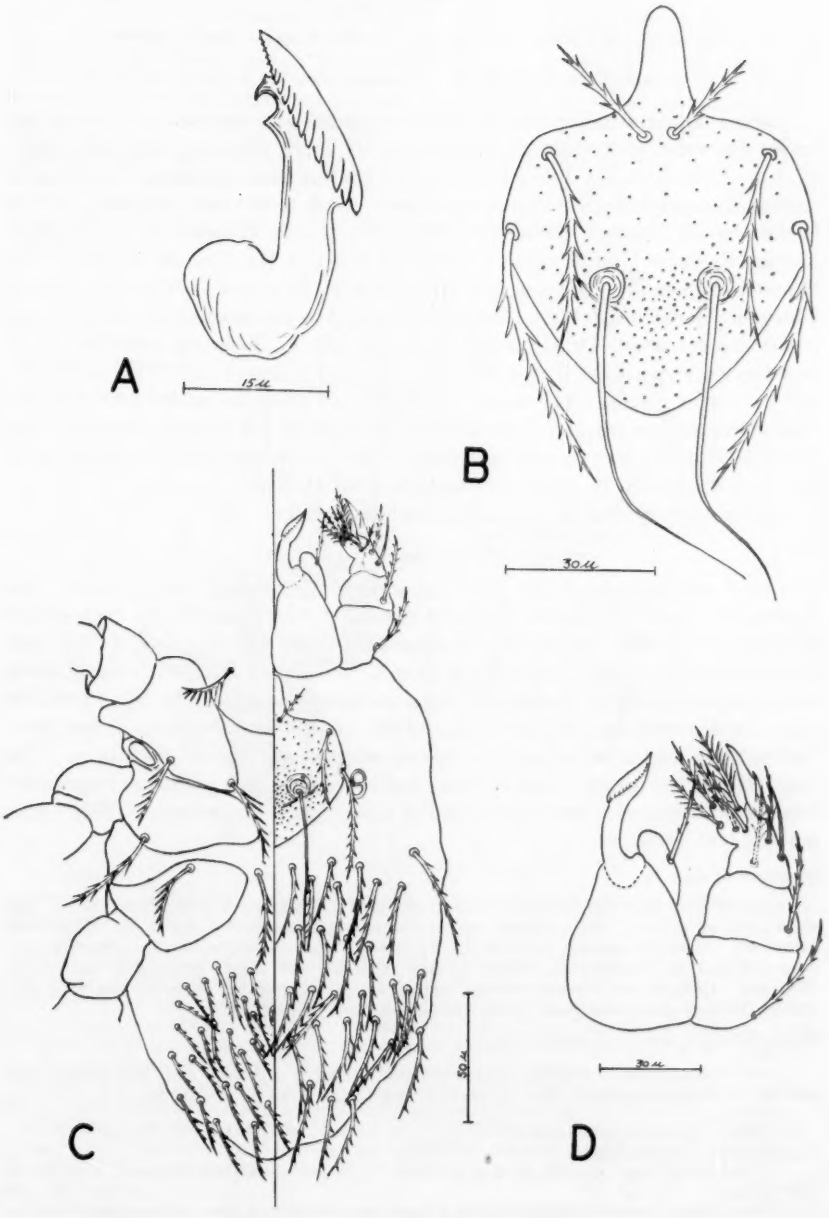
FIG. A. Chelicera, lateral aspect

FIG. B. Scutum

FIG. C. Right—dorsum with scutum and gnathosoma in position. Left—venter. (unengorged).

FIG. D. Gnathosoma, dorsal aspect.

PLATE I



THE *IXODES* TICKS OF CHIROPTERA (IXODOIDEA, IXODIDAE)[1]

DON R. ARTHUR
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and
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During an investigation into *Ixodes* ticks parasitizing mammals it became apparent that existing descriptions of those species which parasitize bats were inadequate in many respects. I therefore obtained for this study specimens from Dr. V. Aellen, Museum d'Histoire Naturelle, Geneva (V.A.), Dr. Gertrud Theiler of the Onderstepoort Research Station (O.R.S.), Mr. Harry Hoogstraal, U. S. Naval Medical Research Unit Number 3, Cairo (H.H.) and Mr. Glen M. Kohls, Rocky Mountain Laboratory, Montana, U.S.A. (R.M.L.) Professor O. Theodor, Hebrew University, Jerusalem (O.T.) allowed me to examine his material, on which he will report in due course. Dr. G. Owen Evans and Mr. E. Browning permitted me to examine specimens in the British Museum (Natural History) (BMNH) collections and were most helpful. The work was carried out when the writer held a Leverhulme Research Award, and is in part a collaborative project between the author and NAMRU–3. For their comments and criticisms of this manuscript I am grateful to Dr. C. B. Philip, Mr. H. Hoogstraal and Mr. Glen M. Kohls.

All measurements in the text are given in millimeters.

Ixodes vespertilionis Koch 1844

Ixodes vespertilionis Koch 1844 has a wide distribution, being known from Europe, the Near and Middle East, and Africa. *I. vespertilionis* was re-described by Neumann (1899), by Nuttall, Warburton, Cooper and Robinson (1911) and by Pomerantzev (1950). Nuttall *et al* (Fig. 279) repeated Neumann's figure of the female capitulum which is drawn from a mounted specimen. In this figure the palps are distorted and the significance of the "salient lateral points" is not clear. Furthermore, certain aspects of the female morphology require elucidation. The description of the male as given by these authors is good; the immature stages have been inadequately described by the earlier authors and Pomerantzev (1950) does not deal with them.

Diagnosis of male

Legs thin, of inordinate length. Coxae unarmed. Capitulum comparatively small, base about as broad as long. Palps clavate, convex dorsally, with numerous long hairs. Hypostome ill defined, practically smooth, indications of few small scalelike teeth distally. Scutum very long and narrow, emargination slight, scapulae rounded and short; cervical grooves faint, diverging. Genital orifice facing second intercoxal spaces. Adanal plates longer than anal plates. Median plate pentagonal, fairly broad, especially posteriorly.

Redescription of female (Figures 1–4)

Body: Unengorged or slightly engorged females 4 long by 2 broad. Legs inordinately long and thin, with tapering tarsi. Hairs of varying lengths numerous on alloscutum.

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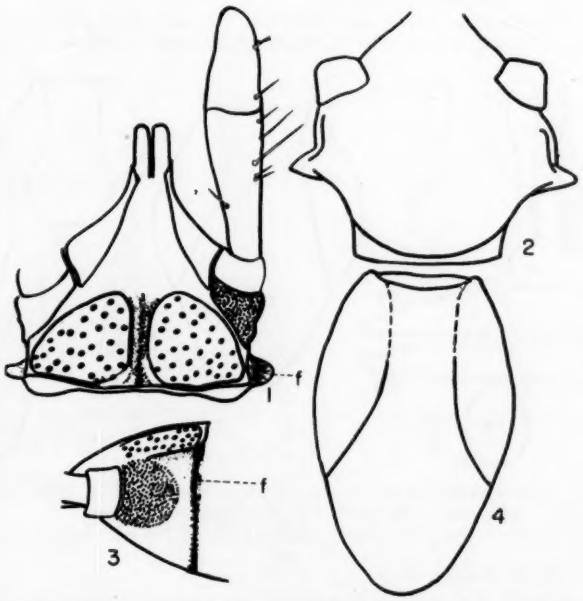
[1] The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Capitulum (Figs. 1, 2 and 3): Basis capituli broad dorsally, triangular, without cornua as such, but posterolateral margins drawn out into salient, broad, "cornua-like" flaps. Basis with lateral margins posterior of insertion of palps either straight or slightly concave; more or less parallel, then converging mesially to base of palps. Posterior to article I an expanse of almost circular membranous tissue, more or less in line with dorsolateral margin of basis. Dorsal ridge and its posterolateral flaps continued around side of basis as sclerotized, vertical, outwardly directed flange (f, in Figs. 1, 3) lying posterior of membranous area at base of palp. Flanges presumably representing "salient lateral points" of earlier authors. Porose areas large, subcircular or subtriangular, somewhat depressed; separated by narrow interval with corrugations, hence a median ridge. Rostrum drawn out to greater extent than figured by Neumann (1899) and repeated by Nuttall *et al* (1911). Palps long and narrow (narrower than figured by Neumann, drawn from mounted preparations). Article II : III :: 1.0 : 0.56; article II, 0.39 mm., article III, 0.22 mm.; long hairs present on outer margin. Basis broad ventrally, convex, convexity terminating along posterior, transverse, curved ridge; posterior margin straight; auriculae absent.



FIGS. 1–4. *Ixodes vespertilionis,* female. 1, capitulum, dorsal; 2, capitulum, ventral; 3, basis capituli, lateral; 4, scutum.

Scutum (Fig. 4): Longer than broad, as 1.0 : 0.6; length 1.76 mm., breadth 1.08 mm.; sides gently curved, broadest about midlength (cf. Pomerantzev, 1950, Fig. 210); posterior border less broad than indicated by Neumann and by Nuttall *et al.* Cervical grooves deeper posteriorly and less distinct anteriorly; extending to posterolateral margins in posterior third of scutum. Hairs sparse, located mainly anterolaterally. Punctations small and shallow, numerous. Scapulae particularly short, rounded, emargination very slight.

Hypostome: Long and pointed, sides converging from base to apex. About 15+ rows with denticles arranged in 3/3 to 4/4 files and approximating to Fig. 33 of Neumann (1899).

Legs: Long and thin, producing "spidery" effect. Surface smooth, with reticulate markings. Tarsus I long; Haller's organ about one-third distance from its tip. Tarsus I, 1.6, metatarsus I, 1.39. Tarsus IV, 1.5, metatarsus IV, 1.36. Length of individual components of leg, however, somewhat variable.

Coxae: Large with surface gently convex; spurs completely absent.

Spiracular plate: Oval, macula anteriorly placed.

Vulva: Between coxae III.

Redescription of nymph (Figures 5–10)

Capitulum: Basis 0.38 long; dorsally triangular, posterior margin straight as in female, flanged posterolaterally and and continued laterally to form dorsoventral ridge. Extensive area of membranous tissue posterior of palp. Surface smooth and shining. Palpi with lateral margins straight, inner margins convex, particularly in distal third; becoming narrower towards base of article II. Article II to article III as 1.0: 0.9. Combined lengths of articles II and III, 0.29; breadth at junction of articles II and III, 0.1. Ventrally a pronounced 'waisting' effect of basis (Fig. 6), but no evidence of auricular projections.

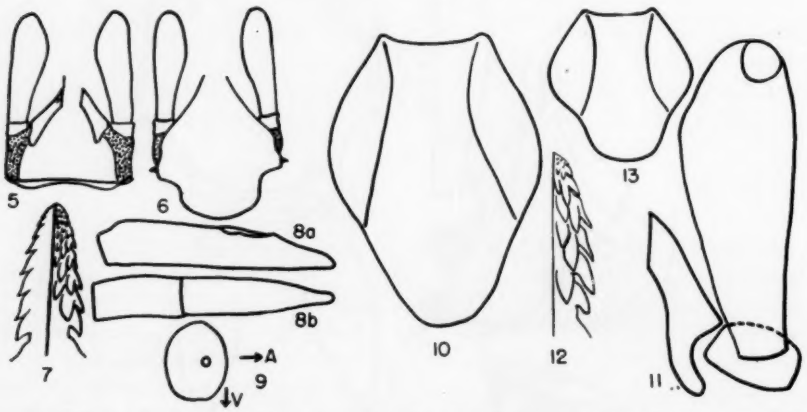
Hypostome (Fig. 7): Relatively shorter and broader when compared with female, pointed apically. Teeth arranged as one basal row of 3/3, succeeded by seven or eight rows of 4/4; length 0.17.

Scutum (Fig. 10): Longer than broad: 0.8 by 0.55. No lateral carinae, cervical grooves distinct but deeper posteriorly, long, first convergent and then divergent, extending to posterolateral margins. Scapulae and emargination as for female. Hairs sparse.

Coxae: Spurs absent, more or less flat. Tarsus I, 0.66, metatarsus I, 0.50. Tarsus IV, 0.69, metatarsus IV, 0.50 (Figs. 8a and b).

Spiracles: Elongate-oval, longest axis transverse, 0.21 by 0.17 (Fig. 9).

Anal grooves: Long and parallel as in female, gently rounded anterior of anus.



FIGS. 5–10. *Ixodes vespertilionis*, nymph. 5, capitulum, dorsal; 6, capitulum, ventral; 7, hypostome, ventral; 8a, tarsus I; 8b, tarsus IV; 9, spiracle; 10, scutum.

FIGS. 11–13. *Ixodes vespertilionis*, larva. 11, left palp; 12, left side of hypostome; 13, scutum.

Redescription of larva (Figures 11–13)

Capitulum: Length, 0.27, triangular, similar to that of nymph and female. Palps attaining greatest breadth nearer to distal than to proximal end (Fig. 11); long with scattered hairs laterally; no sutural line evident between articles II and III; combined length of articles II and III, 0.17.

Hypostome (Fig. 12): Short, relatively broad, length 0.09; outer teeth strong, arranged in one row of 2/2 and five or six rows of 3/3 to tip, median and lateral teeth of about equal strength.

Scutum (Fig. 13): Length, 0.43, breadth 0.41, slightly longer than broad; greatest breadth about midlength; anteriorly with rapidly converging margins; posteriorly from midline margins curved in rather strongly and then more gradually to form broad posterior margin. Cervical grooves long, convergent anteriorly, thence divergent, reaching the posterolateral margins where the latter are concave.

Coxae: As for nymphs; tarsus I, 0.37, metatarsus I, 0.23. Mean relative lengths of individual segments of legs of all stages shown in Fig. 14.

Distribution and hosts

The following records are additional to those given by Neumann (1916):

EUROPE. Gibraltar (Neumann, 1916); Spain (Neumann, 1916; Schulze, 1927; Collado, 1936, 1938, 1948); France (Neumann, 1916; Schulze, 1927); Austria (Neumann, 1916; Nuttall,

1916); England, Ireland, Wales (Neumann, 1916; Nuttall, 1916; Hirst, 1916; MacLeod, 1939; Arthur, 1948, 1953); Hungary (Neumann, 1916; Kotlan, 1921a, b); Germany (Schulze, 1923, 1944; Schulze and Schlottke, 1930); Roumania (Leruth, 1939a); Czechoslovakia (Kolenati, 1856 and 1860 as *Sarconyssus excavatus* from Moravia, but see Neumann, 1910); Bulgaria (Schulze, 1927); Yugoslavia (Nuttall, 1916, cf. Oswald, 1939); Belgium (Bequaert, 1913; Schmitz and Bequaert, 1914; Leruth, 1931, 1939b; Cooreman, 1951); Italy (Neumann, 1916; Tonelli-Rondelli, 1930); Greece (Schulze, 1936; Pandazis, 1947); Sardinia (G. M. Kohls in correspondence with H. Hoogstraal); Netherlands (van Eyndhoven, 1950).

The following are new records from Europe (V.A. collection): from grotte de Ver, Neuchâtel, Switzerland and all collected from *Rhinolophus hipposideros,* 1 unfed nymph, 10.12.1944; 1 unfed female, 18.2.1945; 1 larva, 27.10.1950; 3 fully fed nymph, 1948; 1 fed nymph and larva, 27.10.1950; 1 fed female, 23.10.1949. From grotte de Cotencher, Neuchâtel, Switzerland.

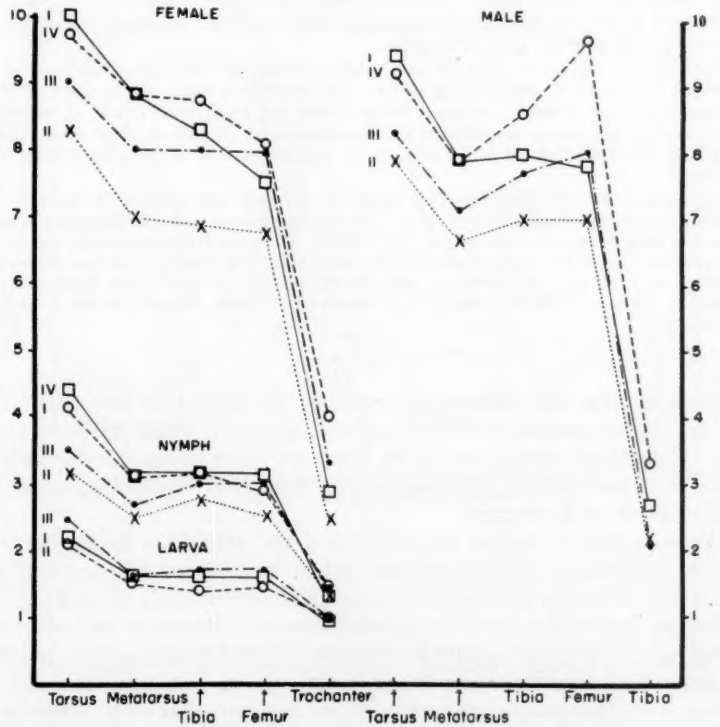


FIG. 14. Relative leg lengths of different stages of *Ixodes vespertilionis.*

1 partly fed nymph, from *Rhinolophus hipposideros,* 18.12.1947; 3 fed and 2 unfed larvae, 1 unfed nymph, from *Plecotus auritus,* 28.1.1951. 1 fed larva from *Myotis daubentoni,* grotte de Pertuis, Neuchâtel, Switzerland, 2.4.1945. 1 partly fed nymph from *Rhinolophus hipposideros,* Soleure, Switzerland, 29.11.1952. 1 gorged female from *Rhinolophus hipposideros,* Ormalingen, Bâle Campagne, Switzerland, 28.7.1949. 1 unfed female from *Rhinolophus hipposideros,* Kristallhöhle, Kobewald, St. Gall, Switzerland, 28.11.1953. 1 gorged female from *Rhinolophus ferrumequinum,* Chatillon Maiche, Doubs, France, –.5.1948. 1 unfed nymph from *Myotis myotis* "galeries souterraines artificielles de Kimmeria, près de Xanthi," Greece, 16.5.1954. 1 unfed female, free-living, grotte de Chrysopighi, Serrès, Greece, 7.5.1954. 1 unfed nymph, "grotte anonyme II près de Mylosavrakhi, près de Sidero-castro," Greece, 11.5.1954. 1 unfed nymph, grotte Katapigui, Areopolis, Greece, 29.11.1952. 1 gorged nymph, "grotte près de St. Jean le Prodrome, Serrès," Greece, 6.5.1954. Within this cave the bats *Miniopterus schreibersi* and *Myotis myotis* are found. 2 males, grotte de Narlidja, Turkey, 19.6.53.

AFRICA. *I. vespertilionis* has been reported from the following localities in Algeria: Rhar el Djema, Ain Amara (Constantine); Beni Add, Ain Fezza (Oran) (Neumann, 1916); Rivet, Guelt es Stel, both records quoted by Senevet (1937); a living female taken from a bat in the Institut Pasteur by Nuttall (Nuttall *et al*, 1911) and a general statement of its occurrence in Algeria (Leruth, 1939b). From Morocco we have the following new records: 2 gorged larvae, 1 gorged nymph from grotte de Sidi bou Knadel, 15 km. north of Rabat, 2.6.1953; 2 gorged females, grotte de Sidi bou Knadel, 15 km. north of Rabat, 9.6.1953 J. B. Panouse *leg.*

Hoogstraal (1954) reported a nymph from an unidentified bat in the Sudan and also (unpublished) from the crater of Mt. Menengai, Kenya. Dr. Theiler (unpublished) recorded it from Uganda and I have identified a male from *Hipposideros caffer*, Lootsberg caves, Cape Province (O.R.S. no. 2621).

RUSSIA. Olenev (1927, 1931) and Pomerantzev (1950) reported its occurrence in Russia and in Northern Iran, and Karpov & Popov (1945) have reported it in western Siberia.

JAPAN. Kohls (in personal communication to Hoogstraal) records it from *Rhinolophus ferrum-equinum*, Sawada, Myiagi, Honshu.

AUSTRALIA. Nuttall *et al* (1911) and Leruth (1939b) referred to the occurrence of *I. vespertilionis* in Australia, and Fielding (1926) has reported it from North Queensland bats.*

There is a lack of detail concerning these records and Hoogstraal (Ticks of the Sudan, in press) has already drawn attention to the improbability of Nuttall & Warburton's specimens being from Australia; he has little hesitation in considering it to be actually a South African record.

Neumann (1916) gave the following genera of European bats as hosts of this tick: *Rhinolophus, Plecotus, Pipistrellus* and *Myotis. Rh. ferrum-equinum* and *Rh. hipposideros* are most frequently parasitized, according to Leruth (1939b). The hosts from which ticks were collected in Switzerland were *Rh. hipposideros, Myotis daubentoni* and *Plecotus auritus.* From Greece, the hosts were *Miniopterus schreibersi* and *Myotis myotis.* In Africa, the hosts thus far recorded are *Pipistrellus kuhlii, Miniopterus schreibersi arenarius, Myotis tricolor ?,* and *Hipposideros caffer.*

BIOLOGY

Biologically, *I. vespertilionis* is interesting. No males have been found on bats, but they wander over the walls of the caves, particularly where the rocks are creviced. Unfertilized females and unfed immature forms occupy similar niches. It seems likely that the fully fed stages secrete themselves between the stones of the caves to digest the blood meal.

A comparison of the data for Switzerland and Macedonia shows that between October and January there is a reasonably high catch of partially and fully gorged ticks, substantiated by the data in the Monograph of Nuttall, *et al.* (1911), while in Macedonia there were a number of unfed nymphs and females in the middle months of the year. The limited material of Leruth (1939b) gives a similar picture and the presence of eight males wandering on the walls in July suggests that possibly feeding takes place mainly during the winter months. It is obvious however that further collections over a continuous period of time are necessary before the seasonal activity of this species can be satisfactorily explained.

This description is based on a single male from South Africa. There is no further information concerning its host or its precise source. The structure of Haller's organ on tarsus I most strongly suggests that it is a species associated with bats (Arthur, in press). It is very probable that this male will prove to be that of an already described female, but it is unsafe at present to attribute it to any known species.

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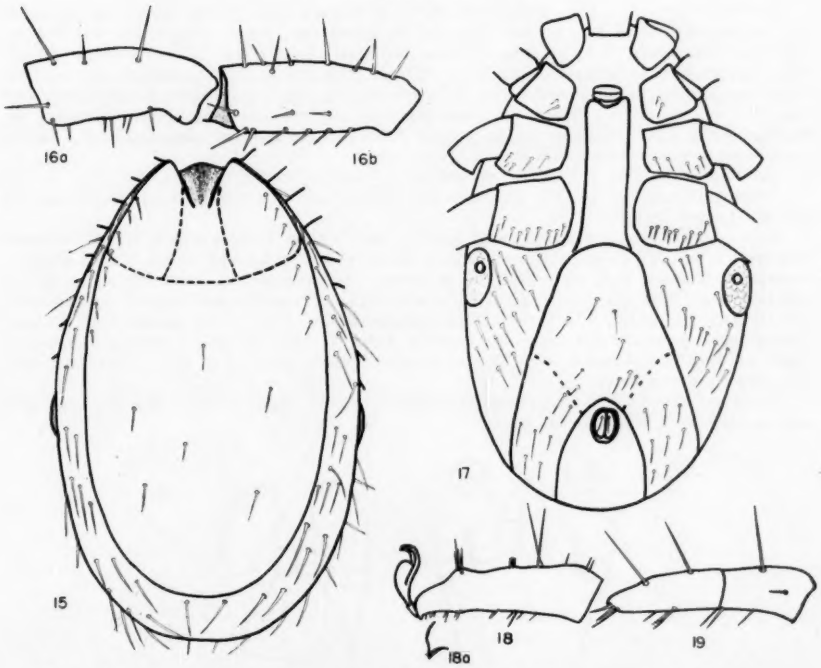
* Ferguson (1925) quotes Nuttall *et al* (1911) that *I. vespertilionis* occurs in Australia.

Ixodes spiculae n. sp.

(Figures 15–23)



FIGS. 15–19. *Ixodes spiculae* n. sp., male. 15, dorsal view; 16a, metatarsus I; 16b, metatarsus IV; 17, ventral view; 18 tarsus I; 18a, apex of tarsus I; 19, tarsus IV.

Description of male

Body (Figs. 15 and 17): Oval, brown, legs stout and long; some of the bristles, notably on the legs and palps, are long and particularly strong. Body length 4.2, greatest width slightly anterior of the spiracles, 2.0. Upper margin of spiracle visible from above.

Capitulum (Figs. 20 and 21): The basis attains its greatest width across the convex dorsal ridge; cornua absent; lateral margins gently curved, converging to the base of the palps and presenting a truncated triangular appearance. Length, including hypostome, 0.35, breadth 0.30. Surface pale yellow and markedly rugose. Palps borne on a broad membranous "palpiger," article I broader than long. Articles II and III without evident suture line between them; combined length of articles II and III 0.29, very broad and globular for the distal three-quarters of their length (greatest width, 0.15) armed with very long stout, straight, and curved spines (Fig. 23). Article IV small, directed downwards. Ventral surface of basis long, lateral margins diverging to the base of the palps, very slightly concave, posterior margin slightly curved. Palps bend downwards; median surface strongly concave basally as seen in ventral view (Fig. 20).

Hypostome (Fig. 22): Short and broad; indented apically with slight lobe arising from each anterior protuberance. Two rows of 2/2 teeth and one row of 1/1 teeth, occupying a third of the distance distally; apical and subapical lateral teeth pointed but thereafter crenulated, proximally no evidence of either lateral or median teeth. Length 0.14.

Scutum (Fig. 15): Twice as long as broad, 3.3 × 1.6; elongate oval, tapering more anteriorly than posteriorly. Color pale brown, translucent in holotype, with gut diverticula showing through. Scapulae rounded, emargination moderately deep but narrow. Cervical grooves deeper anteriorly but fading out towards the posterior limit of the faintly indicated pseudoscutum; greater convexity between the scapulae than elsewhere, where cervical grooves form pronounced valleys. No lateral ridges. Sparsely covered with short white hairs. Surface finely reticulate,

no punctations. Body fold very prominent, wide for the most part; narrowing appreciably at the level of the intercoxal spaces between third and fourth pairs of legs; bearing long, strong, curved bristles.

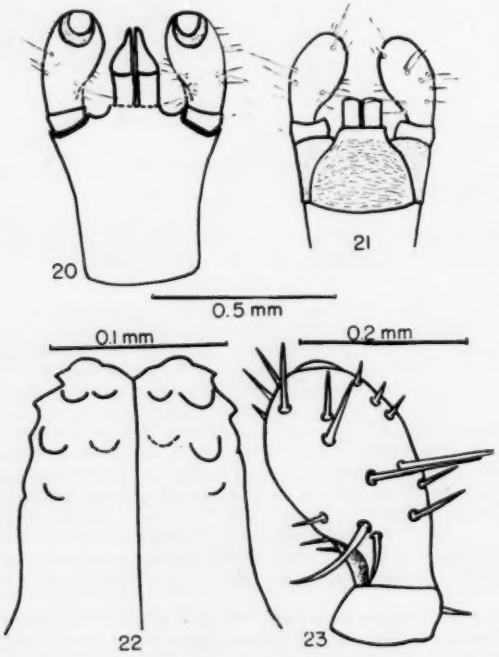
Ventral plates (Fig. 17): Postgenital plate (as distinct from median plate) extends from level of second pair of legs to just posterior of fourth pair, long; rectangular, not heavily sclerotized but marked off by grooves. Median, anal, and adanal plates sharply demarcated from rest of opisthosoma by pronounced grooves. Median plate not strongly sclerotized and no clear distinction between it and adanal plates, latter pigmented. Anal plate more heavily pigmented than other ventral plates. Anal groove rounded anteriorly, (approaching ogival condition), diverging. Long scattered spines on the ventral plates but the epimeral plate behind the fourth coxae more densely covered with similar strong spines.

Genital orifice (Fig. 17): Between anterior margins of second coxae.

Spiracle: Large, egg-shaped; long axis corresponds with long axis of body, macula antero-ventral. Length 0.4 (Fig. 17).

Legs: Large, long and strong, well supplied with spinose bristles which become stronger from leg I to leg IV. Paired spines on ventral surface of leg strong and broad. Tarsal segment appreciably narrower than more proximal segments. Tarsi narrow to claw, with more oblique bend near distal fifth. Length of tarsus I, 0.65; breadth, 0.17; metatarsus I, length 0.67, breadth, 0.23. Tarsus IV, length 0.73, breadth 0.25; metatarsus IV, length 0.72, breadth 0.18. Coxae lacking spurs, generally flat except for coxa I, which is quite strongly convex. Increase in length and breadth of coxae I to IV and in density of bristle cover (Fig. 17). Coxae approach quite close to the midline.

Holotype: deposited in Onderstepoort Research Station, South Africa. No data regarding host or source. O.R.S. No. 2621 (i).

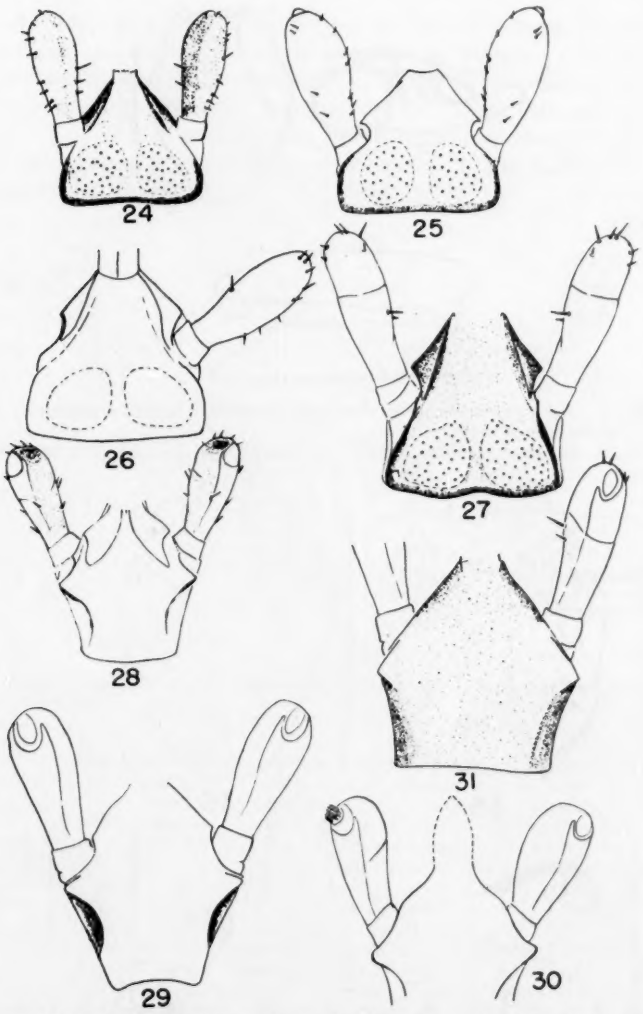


FIGS. 20–23. *Ixodes spiculae* n.sp., male. 20, capitulum, ventral; 21, capitulum, dorsal; 22, hypostome; 23, palp.

Ixodes simplex simplex Neumann, 1906

(Figs. 24–26, 28–30, 33–43)

Ixodes simplex simplex has, apparently, a wide geographical range, but its diag-



FIGS. 24–31. Capitula of females of *Ixodes simplex simplex* (24–26, 28–30) and of *I. simplex africanus* n. subsp. (27, 31). 24, dorsal view, specimen from Banyuls, France (R.M.L. Coll 31988); 25, dorsal view, specimen from near Kameaka, Honshu Japan (R.M.L. coll. 30943); 26, dorsal view, type specimen from Kashmir (BMNH); 27, dorsal view, specimen from Mt. Menengai, Rift Valley (HH 3957); 28, ventral view, specimen from Banyuls, France (R.M.L. coll. 31988); 29, ventral view, type specimen from Kashmir (BMNH); 30, ventral view, specimen from Kameaka, Honshu, Japan (R.M.L. 30943), the posterior region of the basis is obscured due to the distension of the opisthosoma; 31, ventral view, specimen from Mt. Menengai (HH 3957).

nosis in all cases is far from clear. The collection in the BMNH consists of three specimens, each of which is labelled as "type" by Neumann in 1905. All were mounted on cards, and prior to study Dr. G. Owen Evans very kindly resuscitated the specimens with 0.5% tribasic sodium phosphate. Nuttall *et al.* (1911) imply

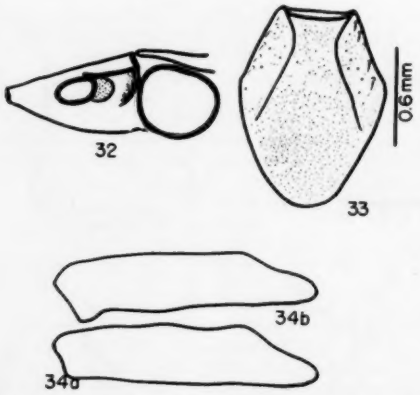
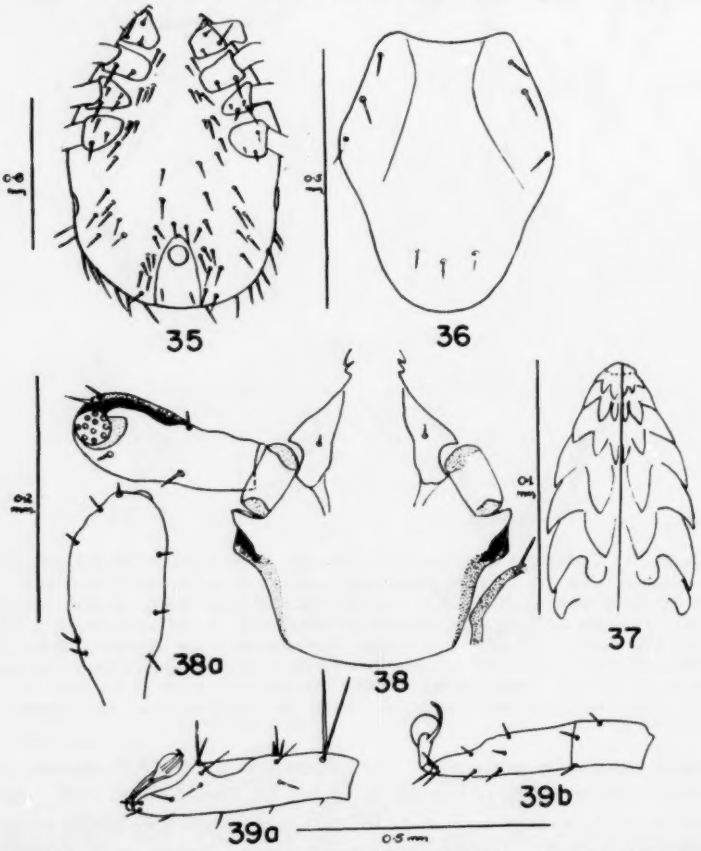


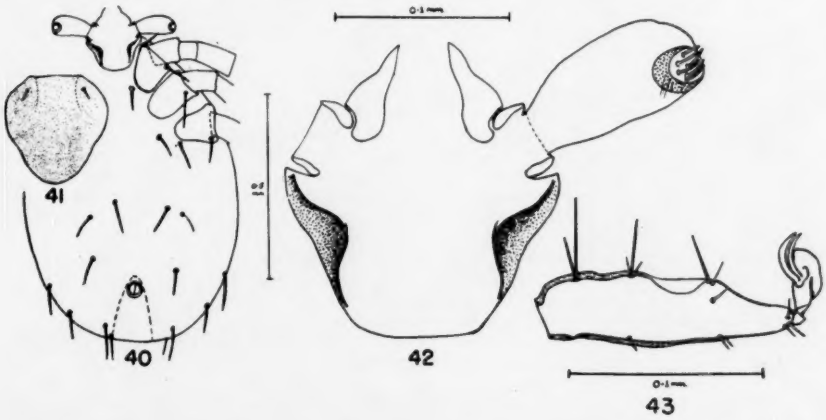
FIG. 32. *I. simplex africanus,* side view, palp removed. Stippling represents the membranous area at the base of the palp.

FIGS. 33–34. *I. simplex simplex,* female. 33, scutum; 34a, tarsus I; 34b, tarsus IV.



FIGS. 35–38. *I. simplex simplex,* nymph. 35, ventral view; 36, scutum; 37, hypostome; 38, basis capituli, ventral view; 38a, palp, dorsal; 39a, tarsus I; 39b, tarsus, IV.

that no hypostome or chelicerae are present on these specimens, but they are in fact present on both the female and the nymph from Shanghai (collection data—"found on bat—*Rhinolophus ferrum-equinum,* Shanghai, R. Swisshoe leg."). One of these type specimens (female) bears no host or locality data and the other is labelled as "? Gaboon, French Congo". Investigation has shown that the latter specimen was removed from *Myotis* (= sp. *Vespertilio*) from Kashmir or a neighboring country and was purchased from E. Gerard in 1873.



FIGS. 40–43. *I. simplex simplex,* larva. 40, ventral view; 41, scutum; 42, basis capituli, ventral; 43, tarsus I.

Material examined: Female and nymph from Shanghai, "found on bat, *Rhinolophus ferrum-equinum,* Shanghai, R. Swisshoe leg."; female from an unknown source; female from Kashmir or a neighboring country: Two females from *Miniopterus s. schreibersi,* Banyuls, France, April, 1937 (R.M.L., no. 31988); one female and three larvae from *Myotis macrodactylus,* Honshu, near Kameaka, Japan, 30.9.1952 (R.M.L., no. 30943); one nymph, from *Miniopterus schreibersi,* Kamakura, Japan, 24.12.1945 (R.M.L., no. 22366); two nymphs, *Miniopterus schreibersi, Myotis myotis,* "grotte près de St. Jean le Prodrome, Serrès, Greece, 6.5.1954" (V.A., no. 27); one nymph "libre grotte de Chrysopighi, Serrès, Greece" (V.A., no. 35); 6 larvae, 4 nymphs, *Miniopterus schreibersi,* "grotte Apano Scola près de Naoussa, Greece, 25.5.1954" (V.A., no. 28); 1 nymph, "libre, grotte Katapigui, Areopolis, Greece, 29.11.1952" (V.A., no. 40); 1 nymph, "origine?, probablement N. Africa, Musèe Zool. Strasbourg" (V.A. no. p. 879); 1 nymph, *Miniopterus schreibersi arenarius,* Ngong, S.E. Mt. Elgon (6000 ft.), Kenya, 11.9.1950 (H. Hoogstraal collection from Chicago Natural History Museum = CNHM); 1 female, 2 nymphs from "bats", Kapretwa, Kitali, Kenya, 7.1939 (O.R.S., no. 2622 ii); 1 nymph, "bats", Kapretwa, Kitali, Kenya, 7.1939 (O.R.S., no. 2622 ii); 1 nymph from *Rhinolophus,* CNHM no. 78471, Anglo-Egyptian Sudan, Equatoria (Katire, 35 miles S.E. Torit), 3.5.1951, host collected by J. S. Owen (ex alc. by H.S.D.); 2 nymphs, *Miniopterus schreibersi,* caves, Sterkstroom, Cape, 6.1944 (O.R.S., no. 2621).

In view of possible confusion I propose that the specimen "from Kashmir or a neighboring country" (BMNH) be elected as the type specimen.

Redescription of female

Body: Color in alcohol preserved specimens varying from yellow to dark brown; scutum, capitulum and legs pale to dark brown. Body oval with distinctive white hairs on alloscutum.

Capitulum (Figs. 24–26, 28–30): Dorsal surface of basis generally flattened in median area, gently curved to base of palps, strongly convex anterolaterally. Lateral margins posterior of palps curved and converging mesad of latter; posterior margins usually straight. No cornua,

posterolateral angles rounded and continuous with dorsoventral flange (Fig. 32). Porose areas consisting of widely scattered pores set in subcircular depression of integument, large separated by narrow interval. Posterior of palps laterally a distinctive crescentic membranous area. Palps short and broad (not as figured by Nuttall *et al.*, 1911), broadest near distal end, narrow proximally; suture line between articles II and III indistinct. In type (from Kashmir) article II 0.17 long; article III 0.15 long. Palps gently curved or straight both laterally, and mesially, more strongly curved towards mesial apex of article III, breadth at junction of articles II and III, 0.14. Hairs few and short. Basis slightly broader than long, length from dorsal ridge to apex of rostrum (excluding hypostome) in Kashmir type 0.37; breadth 0.40. Ventral view, basis pentagonal, posterior border concave in some specimens, slightly convex in others (e.g. Banyuls specimens). Distinct "waist" about one-third distance from posterior margin, profile of "waist" continuous with auricular-like ridges, triangular in form lying behind basal article of palp. Palps flattened on mesial faces except for slight indentation dorsal to article IV. Article I broad, directed antero-laterally.

Hypostome missing in all females except Shanghai specimen (see later) and that from Japan. The latter agrees in essentials with that figured for the nymph (Fig. 37), but, as the specimens belong to the R.M.L., I have not made a preparation of the hypostome.

Scutum (Fig. 33) : Longer than broad, 1.17 by 0.89; broadest slightly anterior of midlength; posterolateral outlines very slightly curved and narrowing appreciably, terminating in curved posterior margin. Anterior of midlength, margin somewhat indented and sclerotization somewhat irregular where convergence begins. Scapulae very small, short, rounded protuberances; emargination ill-defined. Cervical grooves faintly indicated but not obsolete (cf. Fig. 199 of Nuttall *et al.*, 1911, and their monographic text), extending almost to posterolateral margins. No lateral carinae. Color dark brown in Neumann "types", paler in other specimens; glossy; punctations widely separated for the most part. Few short hairs in lateral and anterior fields.

Spiracles: In specimen of unknown source (BMNH), spiracle on right side elongate oval with its long axis transverse to that of body; left spiracle rounded, but somewhat blunter anteroventrally; major axis still transverse. Spiracles of specimens from Kashmir, France, and Japan agreeing with left spiracle of aforementioned specimen.

Legs: Moderately thick and strong.

Coxae: Flat, unarmed. Tarsi parallel-sided for the greater part of their length, but tarsus I beyond Haller's organ tapering gradually to tip. Tarsus I, 0.79 (Fig. 34a), metatarsus I, 0.65. Tarsus IV, 0.78 (Fig. 34b), metatarsus IV, 0.67.

Vulva: Between coxae III.

Anal grooves: Very short; ogival in front, markedly diverging behind.

Description of Nymph

Immature specimens are known in collections but I am unaware of any descriptions or illustrations other than that of the larva given by Bedford (1934). The following descriptions are based on nymphs and larvae from bats examined in collections, from Greece, Africa, and Japan.

Sclerotized parts pale brown; length of gorged specimen, 1.2, breadth 0.86 (Fig. 35).

Capitulum (Fig. 38) : Length, dorsal ridge to tip of hypostome, 0.24; greatest width 0.23. Basis broad, lateral margins short, curved. Cornua absent. Surface slightly convex, smooth, impunctate. Ventrally slightly waisted, sides converging to curved posterior border. Palpi with fairly regular surface, nearly straight laterally, curved mesially; slightly narrowing to base (Fig. 38a). No well-defined auriculae. Hairs few and short. Combined length of palpal articles II and III, 0.16; greatest breadth of 0.07 nearer to the apex than base; sutural lines between articles II and III indistinct.

Hypostome (Fig. 37) : Broadest nearer base; curved profile lines. Denticles arranged from apex to base as 4 rows of 3/3. 3 rows of 2/2, 1 row 1/1. Length, 0.1.

Scutum (Fig. 36) : Length 0.54, breadth 0.42. Widest slightly anterior of midlength. Surface shining, punctations fine and uniformly distributed. Lateral carinae absent. Cervical grooves present and distinct, at first convergent and then divergent in direction of posterolateral margins, but fading out before reaching margin. Hairs few. Scapulae rounded, very short, emargination very shallow.

Coxae (Fig. 35) : As in adults. Tarsus I tapering beyond Haller's organ to apex. Length of tarsus I, 0.44 (Fig. 39a) ; tarsus IV, 0.44 (Fig. 39b).

Description of larva

Body (Fig. 40) : Elongate oval, widest about midlength. Color yellow-brown. As in older stages, opisthosoma bears long hairs. Length of gorged specimen 0.9, breadth 0.62. Basis capituli essentially as in nymph (cf. Figs. 38 and 42). Palps relatively broader than in nymph,

length 0.12, breadth 0.05; straight laterally, curved mesially (Fig. 42). Hypostome missing in all specimens examined.

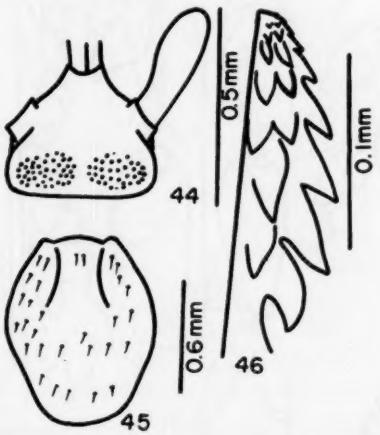
Scutum (Fig. 41): Length 0.3, breadth 0.28. Finely punctate with few hairs. Cervical grooves short, more prominent anteriorly and fading out before reading posterolateral margins.

Anal groove: Ogival.

Coxae: As in later stages, provided with long hairs (Fig. 40); coxa I subtriangular; coxa II long, rounded on its inner face; coxa III subtriangular. *Tarsi* resembling those of later stages, except for somewhat undulate dorsal surface of tarsus I; length 0.13 (Fig. 43).

The Shanghai "Type" Specimen of *Ixodes simplex simplex.*

(Figures 44, 45, 46)



FIGS. 44–46. *I. simplex simplex* female from Shanghai (BMNH). 44, basis capituli, dorsal; 45 scutum; 46, hypostome.

Description of female

The female "type" specimen from Shanghai (BMNH) differs from the other specimens examined in its pale coloring; the scutum is broadly ovate (Fig. 45); the anal groove is rounded anteriorly and has parallel sides. The legs, too, are relatively longer. The porose areas are transversely elongate and depressed (Fig. 44). The question thus arises as to whether these differences are significant or whether we are dealing with a case of intraspecific variation. Because of the small sample, one can not be dogmatic on this point. Within a single species, the scutum is particularly variable and considered alone provides no adequate criterion of subspecies. Likewise, specimens of *I. simplex* from Jerusalem have small subcircular porose areas with a wide interval, but in other respects they agree with the Kashmir specimens.

The Shanghai specimen possesses a hypostome which is broad basally and has a rounded apex. In Fig. 46 the hypostome may be slightly foreshortened as it was drawn *in situ*. Nine lateral teeth are evident on it and the dentition from apex to base is 5 rows of 3/3, 2 rows of 2/2, 1 row of 1/1; apically there is a corona. Length from tip to basal tooth, 0.16. Apart from these differences this specimen agrees in all fundamentals with *I. simplex simplex.*

Description of nymph

Basis of Shanghai nymph strongly constricted ventrally, wider in front, posterior margin straight, postero-lateral margins curved (Fig. 56).

Hypostome (Fig. 49): Broad, rounded apically. Dentition from apex to base, 2 rows of 4/4 teeth, 2 rows of 3/3 teeth, 2 rows of 2/2 teeth, 1 row of 1/1 teeth; the tooth in the basal row rounded apically, remainder pointed. Length 0.11.

Scutum (Fig. 57): Slightly larger and broader than that found in Macedonian, Swiss, and Palestinian material, otherwise closely similar.

Distribution and hosts

Apart from the data given under "material examined", the following records are known: Greece (Schulze, 1937); Japan (Kishida, 1930); Palestine (Theodor; the specimens which

have been examined will be reported on by Prof. Theodor); Belgian Congo (Bequaert, 1931); Kenya (Bequaert, from private communication with Glen M. Kohls, 1.2.1955); South Africa (Bedford, 1934). Zumpt (1950) reported the possibility that larvae and nymphs of an *Ixodes* sp. from the Sterkfontein caves, Witwatersrand, might be *I. simplex*.

(Figs. 27, 31, 47, 58c, 59, 60)

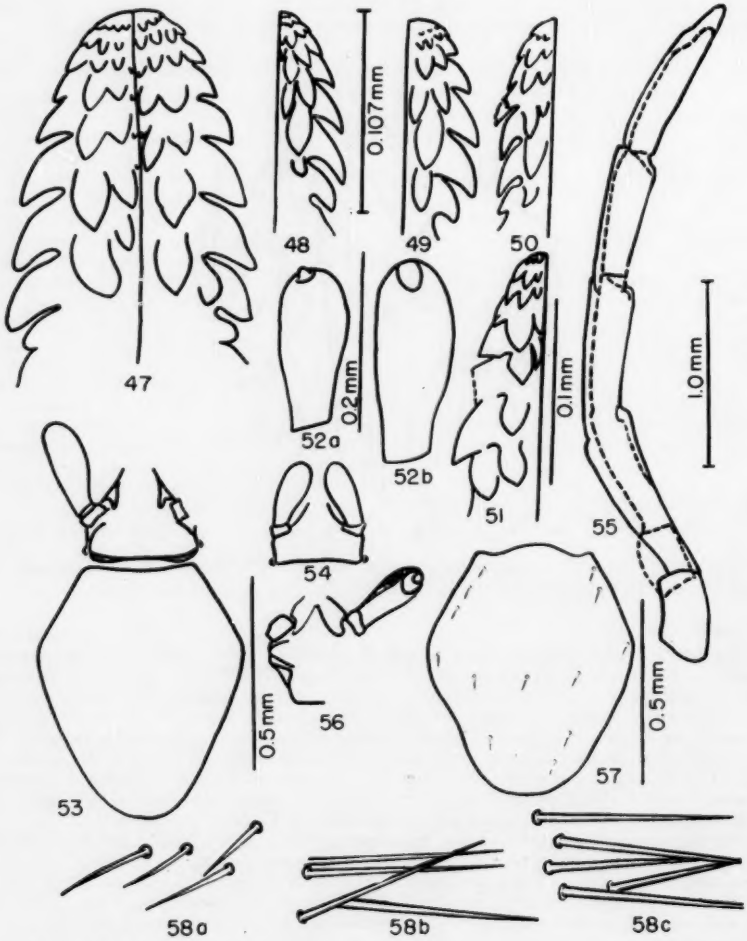


FIG. 47. *I. simplex africanus*, female, hypostome.

FIGS. 48–51. Hypostomes of *I. simplex simplex*. 48, from nymph, Kapretwa, Kitali, Kenya; 49, from nymph, Shanghai; 50, from nymph, Mount Elgon; 51, from female, Palestine.

FIGS. 52a-b. Nymphal palps from *I. simplex simplex* from Kapretwa, Kitali, Kenya (52a); from *Ixodes* sp. *incertae* (ORS coll. 2552) (52b).

FIGS. 53–54. Nymphal capitulum and scutum from *Ixodes* sp. *incertae* (ORS coll. 2552) (53); from *I. simplex simplex* from Kapretwa, Kitali, Kenya (54).

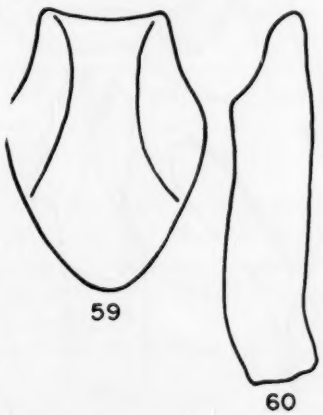
FIG. 55. Leg I of *I. simplex africanus* (thick line) superimposed on leg I of *I. simplex simplex* type from *Kashmir* (broken line).

FIGS. 56–57. *I. simplex simplex*, nymph. Type specimen from Shanghai (BMNH). 56, capitulum; 57, scutum.

FIG. 58a-c. Alloscutal hairs drawn to the same scale and taken from comparable areas of the body. a, from type specimen from Shanghai (BMNH); b, from *I.* sp. *incertae* (ORS coll. 2552); c, from *I. simplex africanus.*

I. simplex africanus new subspecies

The female specimen, which I refer to as the new subspecies *I. simplex africanus,* was collected in the crater of Mt. Menengai, Rift Valley, Kenya (H. Hoogstraal, no. 3957). The specimen was originally identified as *I. simplex* by Dr. Bequaert and Mr. Hoogstraal, but I consider it to merit subspecific rank.



FIGS. 59–60. *I. simplex africanus* female. 59, scutum; 60, tarsus I.

Description of female

Large subspecies, legs long and thick, alloscutum in alcohol-preserved specimens straw-colored, bearing strong, long, white hairs (cf. Figs. 58a, 58b, 58c). Scutum, legs and capitulum pale brown. Female, in partially gorged state, elongate oval. *Capitulum* (Figs. 27, 31, 47): Distinctly longer than broad, length 0.45 mm., breadth across dorsal ridge 0.39 mm. Lateral margins converging more strongly from posterior border to base of palps than in *I. simplex simplex* (cf. Figs. 24, 25, 26, 27). Dorsal ridge distinctly sinuous. Palps longer and relatively narrower, lacking bulbous character of *I. simplex simplex;* length of article II, 0.23 mm., length of article III, 0.13 mm.; hairs few and short. Porose areas very large, set in subcircular depression with well separated pores, some extending to dorsal ridge. Basis ventrally broad, auricular-like extensions not as prominent as in *I. simplex simplex* and waist practically negligible (cf. Figs. 28, 29, 30, 31). *Hypostome:* Length, 0.19 mm.; broad (in Fig. 47 the hypostome has been unduly flattened under the cover glass). Dentition asymmetrical in this specimen, arranged on left side as two rows of 1/1, five rows of 3/3, with an additional minute denticle on mesial side of last four rows, two rows 4/4 to tip; on right side, two rows of 1/1, one row 3/3, one row of 2/2, three rows of 3/3 (again with small mesial denticles), and two rows of 4/4. Hypostomal differences between *I. simplex simplex* (Shanghai specimen), *I. simplex simplex* from Palestine, and *I. simplex africanus* demonstrated by comparing Figs. 46, 47 and 51.

Scutum (Fig. 59): About equally convex anteriorly and posteriorly. Smooth, finely punctate, with few short hairs. Cervical grooves evident but not extending back to posterolateral border. Agreement in shape between the scuta of the two sub-species substantial, and such differences of detail as do exist may well come within normal range of intraspecific variation. Length, 1.35 mm., breadth, 0.9 mm.

Opisthosomatic hairs of *I. simplex africanus* nearly 2.5 times as long as those of *I. simplex simplex.*

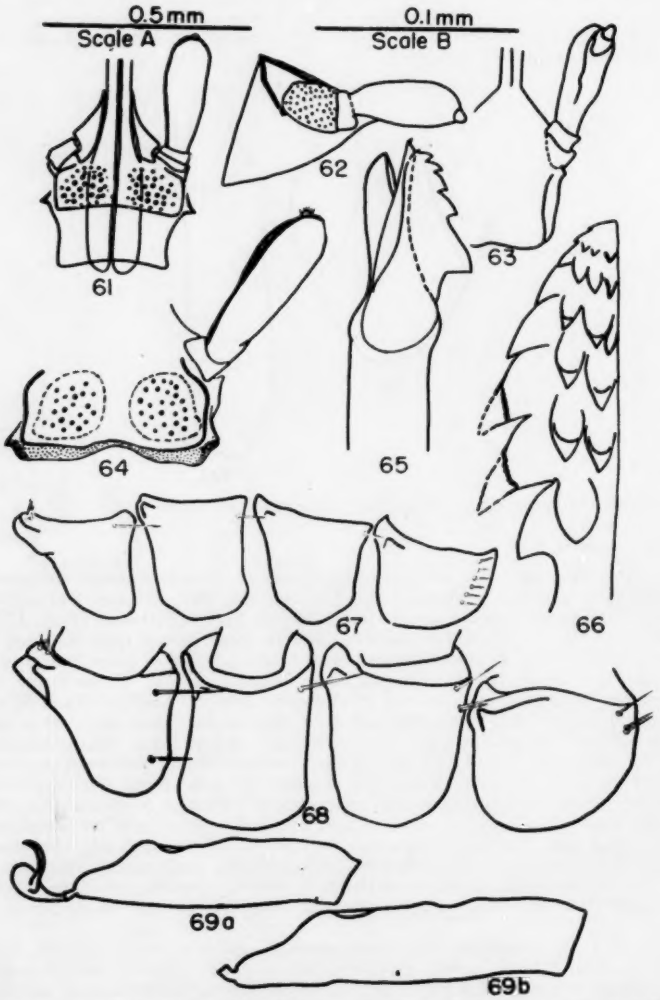
Coxae of African subspecies, as in *I. simplex simplex,* lacking spurs, coxae IV having rounded profile and presenting cupshaped outline; coxae in Shanghai female rectangular. Legs of *I. simplex africanus* longer than any of *I. simplex simplex* examined, and legs of former particularly stout and strong, as depicted in drawing by Hoogstraal (Ticks of the Sudan, in press) and as seen in superimposition of leg I of subspecies *I. simplex africanus* on that of Kashmir

type of *I. simplex simplex* (Fig. 55). Length of tarsus I, 0.89 mm. (Fig. 60), metatarsus I, 0.74 mm. Length of tarsus IV, 0.91 mm., metatarsus IV, 0.78 mm.

Anal groove: Ogival.

Genital orifice: Level with anterior margins of coxae III.

Holotype female: deposited in collection of Chicago Natural History Museum.



FIGS. 61–63. *I. simplex simplex*, capitulum of female from Kapretwa, Kitali, Kenya. 61, dorsal view; 62, side view; 63, ventral view.

FIGS. 64–66. *I.* sp. *incertae*, female from Irene, Pretoria (ORS coll. 2552). 64, capitulum, dorsal. The stippling represents the "cornua-like" flaps. 65, digit; 66, hypostome.

FIGS. 67–68. Coxae I–IV of females of *I. simplex simplex* from Kapretwa, Kitali, Kenya (67) and from *I.* sp. *incertae* from Irene, Pretoria (ORS coll. 2552) (68).

FIGS. 69 a-b, tarsus I of female of *I. simplex simplex* from Kapretwa, Kitali, Kenya (69a) and of *I.* sp. *incertae* (ORS coll. 2552) (69b).

FIGS. 61–64, 67–69a, b, drawn to scale A; FIGS. 65–66, drawn to scale B.

Ixodes sp. incertae

In the collection of ticks sent to me by Dr. G. Theiler is a nymph and female from *Myotis tricolor,* 10.1931, collected in a cave at Irene, Pretoria (O.R.S. no. 2552). Both the nymph and the female are consistently larger than *I. simplex simplex.* Although there is obviously a close affinity with the latter, the differences which make me uncertain are (1) the cornua-like flaps stippled in Figs. 53 and 64 which are reminiscent of those occurring in *I. vespertilionis* and are absent in *I. simplex simplex,* and (2) the very long legs of the Pretoria specimens. This is evident by a comparison of the coxae (Figs. 67 and 68) and of tarsus I (Figs. 69a, 69b). The tarsal and metatarsal lengths approach those of *I. simplex africanus,* being as follows: tarsus I, 0.86 mm., metatarsus I, 0.71 mm.; tarsus IV, 0.89 mm., metatarsus IV, 0.74 mm. The legs however are distinctly stouter than those of *I. vespertilionis* and are intermediate in thickness between those of *I. simplex simplex* and *I. simplex africanus.*

The hypostome (Fig. 66) does not conform to that of *I. vespertilionis* nor to those of known *simplex* specimens, the differences being such as to probably not constitute intraspecific variation. The scutum is not unlike that of *I. vespertilionis* and measures 1.19 mm. long by 0.97 mm. broad. The capitulum and scutum of the Pretoria nymph are figured (Fig. 53). I am hesitant about assigning to these specimens specific or varietal rank until further material is available.

SUMMARY

1. This paper reviews the morphology and distribution of *Ixodes vespertilionis* Koch 1844, with particular reference to the female and the immature stages. New records of its occurrence in Switzerland, Greece, and Morocco are given.

2. A new species, *Ixodes spiculae,* from South Africa is described (host unknown).

3. *Ixodes simplex* Neumann, 1906, is subdivided into two subspecies, *I. simplex simplex,* which has been found in Palestine, Greece, France, Japan, China and Africa and *I. simplex africanus* from the Rift Valley, Kenya. Nymphs and larvae of *I. simplex simplex* are described and figured.

4. A South African form of uncertain status is described and figured but not named. It has affinities with both *I. simplex* and *I. vespertilionis.*

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AN APPARENT CASE OF HUMAN INFECTION WITH THE WHIPWORM OF DOGS, *TRICHURIS VULPIS* (FROELICH, 1789)

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INTRODUCTION

During December 1954 and January 1955, a series of stool specimens from a pediatric patient was forwarded from the U. S. Army Hospital at Fort George G. Meade, Maryland to the Second Army Area Medical Laboratory for routine examination for parasites and ova. These specimens contained adults of the pinworm, *Enterobius vermicularis,* and eggs of a trichurid nematode too large to agree with measurements for eggs of *Trichuris trichura* or *Capillaria hepatica.*

DESCRIPTION OF THE CASE

The patient, a four-year-old Caucasian male, had been brought by his parents to the Fort Meade Hospital exhibiting symptoms of nervousness. He had never left the State of Maryland and had lived with his grandparents on a farm in Severn from the spring of 1953 till October of 1954. To his parents' knowledge, he had never had contact with domestic animals other than three bird dogs, which were kept in a yard where the boy played during his residence on the farm. At the time of this investigation only two of the dogs were living. Fecal specimens from these dogs yielded numerous eggs of *Trichuris vulpis.*

METHODS AND OBSERVATIONS

Following treatment with gentian violet by the Outpatient Department of the Fort Meade Hospital, stools were again forwarded to the Second Army Area Medical Laboratory where examination included straining through a #60 mesh sieve in an attempt to recover adult worms. Though adults of *E. vermicularis* were present, no trichurids were found at this time.

Between December 29, 1954 and July 1, 1955, 16 stool specimens were examined. All 16 stools contained the large trichurid eggs. On May 4, 1955, 3.5 months after treatment, a portion of an adult female *Trichuris,* consisting of the hindbody and about one third of the forebody, was recovered by straining.

This fragment measures 21 mm. in length, hindbody 10 mm. with a maximum width of 0.490 mm. The 11 mm. fragment of forebody varies in width from 0.084 to 0.115 mm., and at the junction of the esophagus and the intestine the specimen is 0.208 mm. in diameter. The anterior rim of the vulva is 0.029 mm. behind this junction. The vulva consists of a fairly prominent cuticular ridge 0.059 mm. in diameter encompassing the round genital aperture. The posterior margin of the ovary is 0.153 mm. from the caudal extremity. The uterus has a maximum width of 0.281 mm. and narrows to form a nearly straight and very muscular vagina 2.5 mm. from the vulva. Diameter of the vagina is approximately 0.120 mm. No vulvar or vaginal spines were observed, though papillae were seen towards the posterior of the vagina. The rectum is 0.364 mm. long, anus subterminal. The eggs of this fragment correspond in size, shape, and general appearance with the

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eggs found in the feces. Measurements taken on 103 of the latter show them to vary from 75 to 90 micra in length (including opercula) and from 33 to 46 micra in width, mean 82 by 38 micra. The adult was examined by Mrs. M. B. Chitwood, A.D. P.R.B., Agricultural Research Center, Beltsville, Maryland, who agreed with a tentative diagnosis of *Trichuris vulpis*. This specimen has been deposited in the U. S. National Museum Helminthological Collection and has been assigned No. 38014.

Some of the eggs, brought to the infective stage in vitro following the methods of Miller (1939, 1941, 1947), were fed to dogs. Each dog received three doses of 2 cc. in butyl chloride prior to experimental feeding of the eggs, but it was later found that larvae of *Trichuris vulpis* were established in the dogs at this time, and that the larvae were resistant to treatment. This phase of the investigation was therefore discontinued.

DISCUSSION

Tentative identification as *Trichuris vulpis* is based on the size of the eggs and the character of the vulva and vagina.

Measurements given for the eggs of *T. trichiura* in the majority of recent textbooks of parasitology vary from 50–56 micra by 21–25 micra. Mönnig (1949) gives the measurements for eggs of *T. vulpis* as 72–89 by 37–40 micra. Though the eggs in question exhibit more variation in size (75–90 by 33–46 micra) than Mönnig has indicated, the variation is centered about his measurements, and the average (82 by 38 micra) is comfortably within the accepted egg size for the dog whipworm.

The vulva is more prominent in this specimen than in specimens of *T. trichiura* examined by the writers, and in this respect, due to the lack of spines, it compares closely with the vulvar regions of previously identified specimens of *T. vulpis* from dogs. Further differentiation from *T. trichiura* is based on the nature of the vagina which corresponds with that of *T. vulpis* in that it is nearly straight and muscular throughout, while the vagina of *T. trichiura* is convoluted.

Schwartz (1926), in comparing whipworms from swine and man, based his taxonomic decisions on egg size, position of the vulva, size of the esophagus, and length and width of the spicules. Chandler (1929) considered certain characters of the male to be most reliable. These characters include spicule length, appearance of the testes, and measurements of the sperm ducts, ejaculatory duct, and cloaca. Clapham (1945) agreed with Chandler but added that certain characteristics of the female reproductive system could also be of aid in specific diagnosis. Chandler (1930) considered egg size to be usually of little value since the measurements for most species lie within a range of 50–60 by 25–35 micra. He cited *T. ovis*, with egg measurements of 70–80 by 30–35 micra, as an exception. The eggs of *T. vulpis* are even larger than those of *T. ovis*, and the close correlation between mean measurements for *T. vulpis* eggs and those in question lead the writers to consider this a valid character. Tiner (1950) used egg size in a key differentiating among species of *Trichuris* from North American rodents. The position of the vulva was disregarded in the present comparison as it is located slightly posterior to the esophageal-intestinal junction in both species. Other characters used by Schwartz and Chandler are not applicable in this case as males were not recovered and the single female specimen is not intact. Mrs. Chitwood (personal communication)

has examined female specimens of *T. trichiura, T. vulpis,* and several species of *Trichuris* from rodents, and considers the vulvar and vaginal characteristics sufficiently consistent to be valid as a diagnostic aid. The size of the hindbody of the present specimen is smaller than is usually reported for *T. vulpis,* but this is very likely a result of development in an abnormal host.

This specimen is distinguished from *Trichuris opaca, T. muris, T. citelli, T. perognathi, T. fossor, T. sylvilagi, T. madisonensis,* and *T. leporis* of rodents and lagomorphs and *T. campanula* of cats by the large egg size; and from *Trichuris ovis* and *T. discolor* of cattle and sheep by the lack of vulvar spines and vaginal convolutions. Two species reported from California rodents, *Trichuris neotomae* Chandler, 1945 from *Neotoma fuscipes* and *T. peromysci* Chandler, 1946 from *Peromyscus californicus,* possess large eggs, straight muscular vaginae, and fairly prominent vulvae. Chandler (1945, 1945) stated that the ovejectors (vaginae) of *T. neotomae* and *T. peromysci* are 1.0 to 1.4 mm. long and 0.7 to 1.4 mm. long respectively. This may serve to distinguish our specimen as its ovejector is 2.5 mm. in length. This measurement was found to be 1.7 to 2.9 mm. in specimens of *T. vulpis* examined for comparison. The authors have recently conducted a survey of rodent parasites in the Fort Meade Area (unpublished data) and have found only *Trichuris opaca* and an unidentified species of *Trichuris* with eggs less than 70 micra in length. Eighty-two specimens of *Peromyscus leucopus* were included in this survey; woodrats are not present in this area. The likelihood of the patient's exposure to eggs of *T. vulpis* over a period of 1.5 years appears to support the tentative placing of the worm with this species. Lack of male specimens prevents a definitive diagnosis. No previous reports of *T. vulpis* occurring in man could be found in the literature.

SUMMARY

A female specimen of *Trichuris,* obtained from the feces of a four-year-old boy who had spent his entire life within Maryland, is tentatively identified on the basis of egg size and characters of the female reproductive organs as *Trichuris vulpis* (Froelich, 1789).

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FOUR TREMATODES FROM KOREAN BATS WITH DESCRIPTIONS OF THREE NEW SPECIES*

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The trematodes reported below are part of a helminth collection made in Korea by J. Knox Jones and Warren D. Thomas, who in 1954 were attached to the 406th Medical Laboratory in Tokyo. The helminths were sent to Dr. H. W. Manter who kindly made the trematode specimens from bats available to me. The study was made under his direction.

Three of the four species are considered new; one in the genus *Prosthodendrium;* two in the genus *Acanthatrium.*

FAMILY PLAGIORCHIIDAE

1. *Plagiorchis vespertilionis* (Müller, 1780) Braun, 1900

Synonyms: P. magnacotylus Park, 1939; *new synonymy.*
P. eptesici Ogata, 1940; *new synonymy.*
(Figs. 1 to 5)

Hosts: Vespertilio superans and *Pipistrellus* sp.

Location: Small intestine.

Locality: Host *V. superans* 7 miles east of Seoul, near the Han River, Korea; *Pipistrellus* sp. from Korea (no other data on collection label).

Museum Specimens: U. S. National Museum Helminthological Collection No. 37256.

Discussion: Plagiorchis vesperilionis was first described from *Plecotus auritus* (Linn., 1758) (*syn. Vespertilio auritus* Linn., 1758) in Europe.

Shtrom and Sondak (1934) reported one specimen from the bat, *Pipistrellus pipistrellus,* in Transcaucasia. They reported a body length of 1.53 mm, and the oral sucker larger than the ventral sucker. Other workers, mainly Braun (1900), von Linstow (1884), and Dujardin (1845) reported a body length of from 4 to 7 mm for this species. On the contrary, Van Beneden (1873) reported a length of only 2 mm. The mature Korean specimens measured from 2.7 to 4.8 mm long; immature specimens from 1.25 to 2.20 mm long. Shtrom and Sondak's (1934) single specimen, 1.53 long, was mature. I believe they were dealing with a closely related species, *P. koreanum* Ogata, 1938, which is mature at least when 1.98 mm long. Measurements of the Shtrom and Sondak specimen agree closely with those of *P. koreanum* Ogata, 1938. I consider the *P. vespertilionis* of Shtrom and Sondak (1934) to be a synonym of *P. koreanum* Ogata, 1938.

A brief diagnosis of *P. vespertilionis,* based on 18 specimens, follows:

Diagnosis: Plagiorchis; body elongate, cuticle spined, body length 2.68 to 4.80 mm; maximum width 0.80 to 1.58 mm. Oral sucker 0.21 to 0.38 mm in diameter. Acetabulum in anterior third of body, 0.25 to 0.42 mm in diameter. Sucker ratio 1 : 0.67 to 1 : 1.39. Pharynx 0.11 to 0.19 mm long by 0.11 to 0.15 mm wide. Esophagus variable in length, sometimes appearing to be absent, at other times very conspicuous depending upon contraction of specimen, when extended, length of esophagus sometimes 1.5 times that of pharynx. Ceca extending into posterior end of body. Genital pore median, midway between posterior edge of cecal bifurcation and anterior edge of acetabulum. Cirrus sac extending from genital pore to mid-ovary or at times to posterior border of ovary. Testes rounded, postequatorial, oblique; anterior testis 0.21 to 0.53 mm in diameter; posterior testis 0.23 to 0.50 mm in diameter. Vitellaria mainly lateral to ceca, overlapping

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ceca mainly in posterior end of body, extending from near posterior tip of ceca to region of acetabulum but sometimes reaching only to acetabulum, sometimes reaching to genital pore at least on one side. Ovary rounded, posterior to acetabulum and dextral; 0.17 to 0.27 mm in diameter. Uterus with descending and ascending loops, intertesticular, joining with male reproductive organs to form very small genital sinus. Eggs 37 by 18 microns. Excretory system not observed.

Considering the variation in size, sucker ratio, and extent of vitellaria, these specimens agree well with descriptions of this species by Braun (1900), von Linstow (1885), and Dujardin (1845).

FAMILY LECITHODENDRIIDAE

2. *Prosthodendrium thomasi* n. sp.

(Fig. 6)

Diagnosis (based on 5 specimens): *Prosthodendrium;* body elongate, 0.224 to 0.356 mm long by 0.126 to 0.147 mm wide. Cuticula apparently aspinose. Oral sucker terminal, 0.070 to 0.071 mm wide. Mid-acetabulum 0.091 to 0.186 mm from anterior end of body. Acetabulum 0.053 to 0.056 mm long by 0.056 to 0.060 mm wide. Sucker ratio 1:0.79 to 1:0.85. Prepharynx short, approximately one-sixth length of pharynx. Pharynx 0.021 to 0.028 mm long by 0.025 to 0.035 mm wide. Esophagus approximately two-thirds as long as pharynx. Ceca very short. Genital pore median, immediately preacetabular. Posterior half of prostatic mass overlapped by anterior half of acetabulum. Prostatic mass 0.031 to 0.042 mm long by 0.049 to 0.049 mm wide. Seminal vesicle obscured by prostatic cells. Testes smooth in outline, slightly irregular in shape, slightly oblique, mostly lateral to posterior half of acetabulum, on either side of body; right testis 0.035 to 0.070 mm long by 0.038 to 0.052 mm wide; left testis 0.034 to 0.052 mm long by 0.042 to 0.049 mm wide. Ovary oval, immediately posterior to acetabulum; 0.035 to 0.049 mm long by 0.024 to 0.035 mm wide. Vitellaria pretesticular, between ends of ceca and testes. Uterus posttesticular, with descending and ascending limbs. Eggs 34 to 35 by 18 to 22 microns. Excretory system not observed.

Host: Vespertilio superans.

Location: Small intestine.

Locality: 6 miles east of Seoul, near Han River, Korea.

Holotype: U. S. National Museum Helminthological Collection No. 37253.

Prosthodendrium thomasi is named after its collector, Mr. W. D. Thomas.

Discussion: Dubois (1955) revised the sub-genus *Prosthodendrium* and recognized nineteen species and three sub-species. Of these, the following appear to be most similar to *P. thomasi: P. macnabi* Macy, 1936; *P. dinanatum* (Bhalerao, 1926) Dollfus, 1931; *P. ascidia* (Van Beneden, 1872) Dollfus, 1931; *P. ascidia navicula* Macy, 1936; *P. urna* (Looss, 1907) Dollfus, 1931; and a species considered inquirienda by Dubois (1955), *P. liliputianum* (Travassos, 1928) Dollfus, 1937.

P. macnabi differs from *P. thomasi* in body length (1.0 to 1.19 mm as compared with 0.224 to 0.356 mm); sucker ratio (1:1 as compared with 1:0.79 to 0.85); ceca reaching almost to testes; and prostatic mass anterior to the acetabulum.

P. dinanatum differs from *P. thomasi* in body length (0.57 to 0.59 mm as compared with 0.224 to 0.356 mm); in sucker ratio (1:0.5 as compared with 1:0.79 to 0.85); mainly prececal vitellaria; eggs 22 to 23 by 12 to 14 microns (compared with 34 to 35 by 18 to 22 microns).

P. ascidia differs from *P. thomasi* in body length (0.780 to 0.936 mm as compared with 0.224 to 0.356 mm); eggs 22 to 30 by 13 to 15 microns (compared with 34 to 35 by 18 to 22 microns).

P. ascidia navicula differs from *P. thomasi* in body length (0.360 to 0.565 mm as compared with 0.224 to 0.356 mm); in sucker ratio (approximately 1:0.66 as compared with 1:0.79 to 0.85); ceca reach almost to testes; eggs 18 to 20 by 10 to 12 microns (compared with 34 to 35 by 18 to 22 microns).

P. urna differs from *P. thomasi* in body length (0.50 to 0.55 mm as compared with 0.224 to 0.356 mm); eggs 24 to 26 by 13 to 15 microns (as compared with 34 to 35 by 18 to 22 microns).

P. liliputianum differs from *P. thomasi* in sucker ratio (approximately 1 : 1.38 as compared with 1 : 0.79 to 0.85); vitelline position (mainly prececal as compared with mainly postcecal).

3. *Acanthatrium macyi* n. sp.
(Fig. 7)

Diagnosis (based on three specimens): *Acanthatrium;* cuticula spined; body length 0.81 to 0.96 mm; maximum width 0.42 to 0.48 mm. Oral sucker terminal, 0.072 to 0.098 mm in diameter. Pharynx 0.042 to 0.049 mm long by 0.035 to 0.049 mm wide. Esophagus 0.120 mm long when extended; ceca extending to anterior border of testes, ending about one-third body length. Acetabulum 0.043 to 0.056 mm wide; mid-acetabulum 0.309 to 0.343 mm from anterior end of body. Sucker ratio 1 : 0.47 to 0.78. Genital pore median, immediately anterior to acetabulum. Testes rounded, lateral, slightly preequatorial; 0.08 to 0.14 mm long by 0.08 to 0.13 mm wide. Prostatic mass intercecal, median, beween posterior edge of cecal junction and mid-testicular level; 0.168 to 0.189 mm long by 0.175 to 0.203 mm wide; membrane surrounding prostatic mass and also vesicula seminalis and genital atrium. Atrium armed with seven bluntly tipped spines, 0.007 to 0.008 mm long; located anteriad of utero-atrial junction. Ovary 0.126 mm long by 0.098 mm wide, equatorial, slightly sinistral, almost touching left testis. Vitellaria pretesticular, mainly lateral to ceca, composed of small irregular follicles. Uterus filling most of hindbody posterior to testes. Receptaculum seminis uterinum noticeable only in sections. Eggs 28 by 14 microns.

Host: Pipistrellus sp.
Location: Small intestine.
Locality: Korea (No other data on collection label.)
Holotype: U. S. National Museum Helminthological Collection No. 37254.
A. macyi is named after Dr. R. W. Macy of Reed College, Portland, Oregon.

Discussion: Ten species and 1 sub-species have been named in the genus *Acanthatrium* Faust, 1919. *A. alicatai* Macy, 1940; *A. chosenicum* (Ogata, 1940) n. comb. (synonym *Lecithodendrium chosenicum* Ogata, 1940); *A. eptesici* Alicata, 1932; *A. microcanthium* Macy, 1940; *A. mollosidis* Martin, 1934; *A. nycteridis* Faust, 1919; *A. nycteridis plicati* Bhalerao, 1926; *A. pipistrelli* Macy, 1940; *A. oregonense* Macy, 1939; *A. ovale* Yamaguti, 1939; *A. sphaerula* (Looss, 1896) Faust, 1919. *Acanthatrium macyi* differs from *A. alicatai* in the following characters: The body is longer in proportion to width (approximately 1 : 0.5 as compared with 1 : 0.84 to 0.95); the eggs differ in size and shape (28 by 14 microns as compared with 29 to 32 by 17 to 18 microns). *A. macyi* differs from *A. pipistrelli* as follows: In having much shorter atrial spines (7 to 8 microns as compared with 25 microns); a longer esophagus; and different sucker ratio (1 : 0.47 to 0.78 as compared to 1 : 0.93 to 1.12).

4. *Acanthatrium jonesi* n. sp.
(Figs. 8, 9)

Diagnosis (based on six specimens): *Acanthatrium;* body pear-shaped, cuticula aspinose; body length 0.46 to 0.65; maximum width 0.28 to 0.32 mm. Mid-acetabulum 0.21 to 0.28 mm from anterior end of body. Acetabulum 0.056 to 0.067 mm long by 0.056 to 0.066 mm wide; with two broad lobes on posterior lip. Oral sucker terminal, 0.070 to 0.077 wide Sucker ratio 1 : 0.8 to 0.9. Prepharynx absent. Pharynx 0.028 to 0.042 mm long by 0.028 to 0.042 mm wide. Esophagus approximately three-fourths length of pharynx. Ceca extending almost laterally and terminating slightly in front of testes near level of anterior edge of acetabulum. Genital atrium with numerous minute spines at its anteriormost end; spines 7 to 12 microns long. Prostatic mass mainly to right of mid-line, between testes, extending to posterior border of acetabulum, 0.049 to 0.105 mm long by 0.049 to 0.091 mm wide; seminal vesicle coiled. Testes lateral, on either side of acetabulum; 0.056 to 0.106 mm long by 0.56 to 0.112 mm wide. Vitellaria large, irregular in shape, lateral and anterior to ceca, not confluent anteriorly, extending from level of esophagus to ends of ceca. Vitelline ducts extending posteriorly, mesially, and ventral to prostatic

mass, joining near equator. Ovary overlapping prostatic mass slightly, dextral, immediately posterior to acetabulum; 0.049 to 0.084 mm long by 0.035 to 0.077 mm wide. Uterus mainly posttesticular descending on right side and ascending on left side of body and entering genital atrium on left side. Eggs (Fig. 9) thick-shelled, with operculum; 21 to 31 to 14 by 20 microns.

Host: Vespertilio superans.

Location: Intestine.

Locality: 7 miles east of Seoul, near the Han River, Korea.

Holotype: U. S. National Museum Helminthological Collection No. 37255.

A. jonesi is named after its collector, J. Knox Jones.

Discussion: A. jonesi differs from all other species in the genus *Acanthatrium* Faust, 1919 in possessing acetabuluar lobes. It closely resembles *A. ovatum* Yamaguti, 1939 from a different host, *Rhinolophus ferrum-equinum nippon* Temm., in Kyoto, Japan, but differs from that species in having an equatorial acetabulum (as compared with postequatorial) and possessing lobes.

A. jonesi is also closely related to *A. chosenicum* (Ogata, 1940) differing from it as follows: The testes are more posteriorly located (testes lateral to acetabulum as compared with the posterior end of testes approaching the anterior end of acetabulum), the ceca are more voluminous and do not overlap the genital atrium; the spines of the genital atrium are arranged differently (at anterior end of atrium as compared to covering most of the atrial wall); the acetabulum has lobes; and finally the body is smaller (0.46 to 0.65 mm as compared with 1.25 mm.)

THE STATUS OF ACANTHATRIUM FAUST, 1919 AND RELATED GENERA

Dollfus (1937) discussed at some length the confusion then existing among certain genera and sub-genera (*Lecithodendrium* Looss, 1896; sub-genus *Lecithodendrium* Odhner, 1911; *Acanthatrium* Faust, 1919; *Mesodendrium* Faust, 1919; *Prosthodendrium* Dollfus, 1931; and the sub-genus *Paralecithodendrium* Odhner, 1911. Dollfus's move in establishing the genus *Prosthodendrium* and naming *L. linstowi* (syn. *L. ascidia* of Looss, *nec* Van Beneden) appears to be sound for the following reasons: When Van Beneden (1873) named *"Distomum ascidia"* from various bats in Belgium, he described and figured it as a species with vitellaria far forward in the pharynx region. Von Linstow (1884) recorded what he thought to be the same species but in his specimens the vitellaria were posttesticular. Looss (1894) also identified species with posttesticular vitellaria as being *"D. ascidia."* He later (1898, p. 455) expressed the belief that Van Beneden had mistaken gland cells as vitellaria. This view was apparently shared by Lühe (1909).

The genus *Lecithodendrium* was named by Looss 1896 (p. 86) without designation of type species. In 1899, Looss designated *"D. ascidia* Van Beneden, 1873" as type species. As Dollfus (1937) has pointed out in detail, Looss clearly indicated at various times that what he considered to be *"D. ascidia* Van Beneden" was the *D. ascidia* Van Beneden of Von Linstow, a misidentification.

Dollfus (1931) named the genus *Prosthodendrium* for the true *D. ascidia* Van Beneden, 1873, accepting *Lecithodendrium* Looss, 1896 for species with posttesticular vitellaria. From the viewpoint of taxonomy, there are clearly two genera. The problem of nomenclature is more difficult. Dollfus explained his solution in detail (Dollfus, 1937). While the original Rules of Nomenclature appear to establish the designated type species as dictating the characteristics of a nominal genus regardless of what was in the mind of the author, changes adopted at the Copenhagen meetings give support to Dollfus. The Copenhagen Decisions on Zoological

Nomenclature (1953) include a statement (p. 69; paragraph 168 (1)) that a generic name based on a nominal species as type but indicated to refer actually to a taxon erroneously identified with the nominal species (in this case the taxon is the *"D. ascidia"* of Von Linstow), the type species is not to be the one cited by the author but the species misidentified by him. This recommendation appears to support the view of Dollfus that the *"D. ascidia"* of Von Linstow should be the type species of *Lecithodendrium*. The other possibility, that *Lecithodendrium* is a genus with anterior vitellaria, would necessitate a reversal of the two genera as used by recent authors (e.g. Macy, Dubois, Yamaguti), would cause numerous changes in names, and would compound the present confusion.

Dollfus (1937; p. 13) reduced *Mesodendrium* Faust, 1919 to synonymy with *Lecithodendrium* (*sensu* Dollfus).

Paralecithodendrium Odhner, 1911 is a sub-genus of *Prosthodendrium*.

In 1919, Faust named the genus *Acanthatrium* for lecithodendrids with spines in the genital atrium, with *A. nycteridis* as type species. This genus possesses pretesticular vitellaria and is differentiated from *Prosthodendrium* only by its spinuous genital atrium.

Lecithodendrium japonicum Yamaguti, 1939 has posttesticular vitellaria but also spines in the genital atrium. Skarbilovich (1948)[1] moved this species to the genus *Acanthatrium*, naming for it the sub-genus *Mesothatrium*. It is like *Acanthatrium* in its spined atrium, but differs in its posttesticular vitellaria. Since this latter character appears to be of generic value in this group of trematodes, the subgenus *Mesothatrium* should be raised to generic rank. *Acanthatrium* (*Mesothatrium*) *japonicum* (Yamaguti, 1939) Skarbilovich, 1947 becomes *Mesothatrium japonicum* (Yamaguti, 1939) n. comb., and the type species of *Mesothatrium*.

The genera *Lecithodendrium*, *Mesothatrium*, *Acanthatrium* and *Prosthodendrium* may be keyed as follows:

Posttesticular vitellaria
- Aspinose genital atrium ..... *Lecithodendrium* Looss, 1896
- Spinose genial atrium ..... *Mesothatrium* Skarbilovich, 1948

Pretesticular vitellaria
- Aspinose genital atrium ..... *Prosthodendrium* Dollfus, 1931
- Spinose genital atrium ..... *Acanthatrium* Faust, 1919

SUMMARY

1. *Plagiorchis magnacotylus* Park, 1939 and *Plagiorchus eptesici* Ogata, 1940 (Plagiorchiidae) are considered synonyms of *Plagiorchis vespertilionis* (Müller, 1780) Braun, 1900.

2. *Plagiorchis vespertilionis* of Shtrom and Sondak (1934) is considered a synonym of *Plagiorchis koreanum* Ogata, 1938.

3. Three new species in the family Lecithodendriidae are reported: *Prosthodendrium thomasi* and *Acanthatrium jonesi* from the Korean bat, *Vespertilio superans;* and *Acanthatrium macyi* from the Korean bat, *Pipistrellus* sp.

4. *Lecithodendrium chosenicum* Ogata, 1940 is transferred to the genus *Acanthatrium* and becomes *Acanthatrium chosenicum* (Ogata, 1940) n. comb.

5. The sub-genus *Mesothatrium* Skarbilovich, 1947 is elevated to the rank

[1] According to Skarbilovich (1948) the date was 1947 although no reference for this date could be found.

of genus. *Acanthatrium* (*Mesothatrium*) *japonicum* (Yamaguti, 1939) Skarbilovich, 1947 becomes *Mesothatrium japonicum* (Yamaguti, 1939) n. comb. and is the type species of *Mesothatrium*.

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Explanation of Plate

All figures were drawn with the aid of a camera lucida The projected scale has value in millimeters indicated in each figure. Abbreviations used: ac, acetabulum; as, atrial spine; c, cirrus; e, egg; ga, genital atrium; gp, genital pore; isv, internal seminal vesicle; ov, ovary; pm, prostatic mass; t, testis; ut, uterus; vt, vitellaria.

FIG. 1. *Plagiorchis vespertilionis,* ventral view.

FIG. 2. *Plagiorchus vespertilionis,* ventral view of cirrus sac. Acetabulum dotted in to show its location.

FIG. 3. *Plagiorchis vespertilionis,* vitellaria entirely posterior to acetabulum.

FIG. 4. *Plagiorchis vespertilionis,* vitellaria on right side of body extending to anterior border of acetabulum.

FIG. 5. *Plagiorchis vespertilionis,* vitellaria on both sides of the body extending to mid-acetabular level.

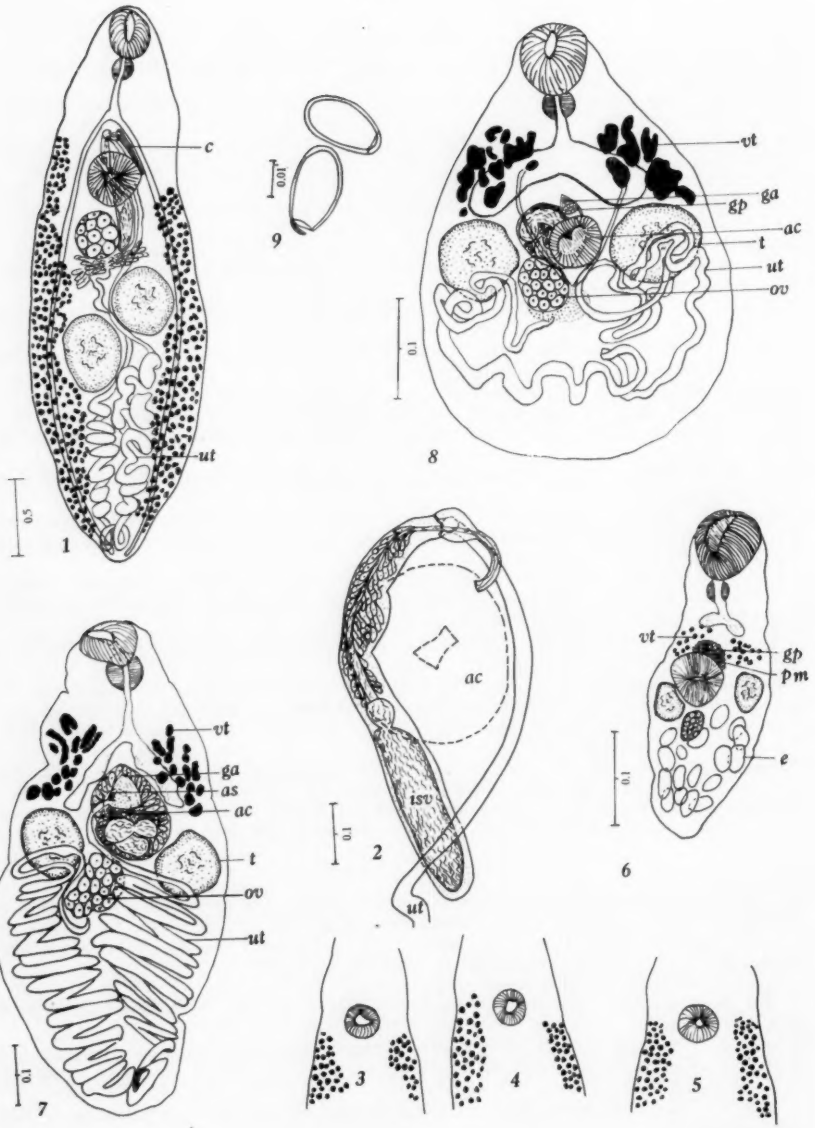
FIG. 6. *Prosthodendrium thomasi,* ventral view.

FIG. 7. *Acanthatrium macyi,* dorsal view. Acetabulum dotted in to show relationship.

FIG. 8. *Acanthatrium jonesi,* ventral view.

FIG. 9. *Acanthatrium jonesi,* eggs.

PLATE I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RESEARCH NOTE

THE CHROMOSOMES OF *HYDATIGERA TAENIAEFORMIS* AND *TAENIA PISIFORMIS*

As part of a study (supported in part by the U. S. Atomic Energy Commission Contract No. AT(40-1) 1749) of the effects of radiation upon cestodes, we considered the possible usefulness of chromosome aberrations as a measure of radiation damage. Because of the small size of the chromosomes and the peculiar technical difficulties of this material, we cannot rely on chromosomal indications; nevertheless we wish to report what we know of the chromosomes of *Hydatigera taeniaeformis* and *Taenia pisiformis*. Since the early inconclusive work of Harman (1913, J. Morph. **24**: 205–242) there has been no published report on these chromosomes.

All ordinary cytological techniques were tried but only one, Schreiber's (1954, Stain Tech. **29**: 285–291) feulgen-aceto-carmine method, gave good results. The chromosomes of *Hydatigera taeniaeformis* number 16 diploid, and range in size from about 1 to about 2 microns. In meiosis, they form eight bivalents, with one, seldom two, chiasmata each (see Figs. 1–4, 8). The chromosomes of *Taenia pisiformis* number 20 diploid, and are somewhat larger than those of *H. taeniaeformis*. They form bivalents regularly, some of which may have as many as four chiasmata (see Figs. 5–7, 9).

DESCRIPTION OF FIGURES

FIGS. 1–4, 8, the chromosomes of *Hydatigera taeniaeformis*, showing, consecutively, mitosis, spermatocyte diakinesis, spermatocyte metaphase I, spermatocyte metaphase II, and a tentative idiogram of the mitotic chromosomes.

FIGS. 5–7, 9, the chromosomes of *Taenia pisiformis*, showing, consecutively, mitosis, spermatocyte pachytene (an entire nucleus, with the same bivalents drawn separately) oocyte metaphase (with the spiral, ribbon-like sperm nucleus at right) and tentative idiogram of the mitotic chromosomes. All figures drawn with the aid of a camera lucida, at the same magnification.

These chromosome numbers are among the higher reported so far for cestodes (Jones, 1945, J. Parasit. **31**: 213–235; Jones, 1951, Trans. Amer. Micros. Soc. **60, 3**: 272–3).—ARTHUR W. JONES, *Department of Zoology and Entomology, The University of Tennessee, Knoxville,* AND HONORICO CIORDIA, *U. S. Department of Agriculture Animal Disease and Parasite Research Branch, Experiment, Georgia.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Forty-fifth Council Meeting, Atlanta, Georgia, December 27, 1955

The meeting of the Council of the American Society of Parasitologists was called to order by President Clay G. Huff at 7:45 P.M., Tuesday, December 27, 1955, in Room "Panel C", Dinkler Plaza Hotel, Atlanta. In addition to President Huff, President-Elect E. B. Cram, Past-Presidents E. E. Becker, H. E. Meleney, H. W. Stunkard, and W. H. Wright, Vice-President G. F. Otto, the Secretary, and the following members of Council were present: W. S. Bailey, E. E. Byrd, R. M. Cable, A. O. Foster, G. R. LaRue, A. McIntosh, D. V. Moore, L. O. Nolf, and L. A. Stauber. L. Jacobs was also present during a portion of the meeting.

The regular order of business was taken up.

I. Reports of Officers and Members of the Council.

1. *President* (*C. G. Huff*): No report. As presiding officer, he appointed A. O. Foster, D. V. Moore, and L. A. Stauber to act as the auditing committee for all financial reports to be submitted.

2. *President-Elect* (*E. B. Cram*): No report.

3. *Vice-President* (*G. F. Otto*): No report.

4. *Secretary* (*A. C. Walton*): As of December 27, 1955, the Secretary's records show that there are 941 names on the rolls of the Society of whom 94 are residents of foreign countries (23 from Canada). Of these 941, 4 are listed as Life Members, 11 as Honorary Foreign Members, 875 as Active Members in good standing, 1 as Inactive (Armed Services), 22 as delinquent in 1955 dues, and 28 as delinquent in both 1954 and 1955 dues. These 28 will be dropped from the rolls as of January 1, 1956, unless their back dues are paid up before that date. Of the 847 members residing in the U.S.A., 15 have over-seas addresses. Since the last meeting, notices of the death of 6 members (one Foreign Honorary, two Charter, and three Active) have been received; six Active Members have resigned; and 24 were dropped as of January 1, 1955 for non-payment of dues. In the same period the names of 65 individuals have been added to the list of Active Members. Later in this session the names of 19 additional candidates will be presented for Council action. Up to the present, 2008 applications for Active Membership have been acted upon, of which 22 have been re-elections. Of the 321 Charter Members, 67 have maintained continuous membership (four are now Life Members) and four others have been re-elected after periods of non-membership. Of the first 1000 members elected (1925–1940), over one-fifth are still supporting our Society.

During the past year the Secretary by direction of the President has handled the reports of the two Special Committees appointed by the Presiding Officer of the moment to bring in suggestions for membership on the Editorial Committee of the Journal of Parasitology. The necessity arose because of the resignation of Dr. LaRue and his committee associates before the end of their regular term (December 31, 1958), and the later resignation of one of the newly elected Committee members before the beginning of his term. The first Special Committee, appointed at Memphis by President Philip, consisted of Drs. Stoll and Stunkard as co-chairmen and Dr. Huff. This committee on May 10, 1955, formally reported that Drs. Coatney, Manter and Travis were their nominees for positions on the Editorial Committee of the Journal of Parasitology, and further recommended that Dr. Coatney be designated as Chairman. This report was accepted by mail ballot of Council in July and by a second ballot of the same date the three men were elected and Dr. Coatney designated as Chairman. The election was for a term of five years ending December 31, 1960. Because of health reasons, Dr. Coatney was forced (August 9, 1955) to resign from all duties connected with the Editorial Committee. President Huff then appointed a second Special Committee (Dr. Herman, Chairman, and Drs. Sarles and Wharton) on August 11 to make further recommendations. This committee on August 13 suggested the names of Drs. Jacobs, Manter and Travis, with the recommendation that Dr. Jacobs be selected to act as Chairman of the Editorial Committee. By mail ballots of September 11, 1956, Council voted to accept the recommendations and also to elect Dr. Jacobs to membership on the Editorial Committee and to serve as the Chairman. At the same time Council re-affirmed the election of Drs. Manter and Travis as the other members of the Committee. Dr. Jacobs is to represent the field of Protozoology, Dr. Manter the field of Helminthology, and Dr. Travis the field of Entomology, in accordance with the requirements as set forth in our Constitution.

The new Committee is to serve for five years, beginning with January 1, 1956 (Vol. 42 of the Journal) and continuing through December 31, 1960 (Vol. 46). These three men have accepted the responsibilities involved and are already at work on the Journal for 1956.

The expenses of the Secretary's office during the past fiscal year have amounted to $230.33,

but through the generosity of a friend, only $130.33 of this has been charged to the Society treasury. A summary of the monies in the hands of the Secretary is as follows:

Balance on hand, November 1, 1954	$ 46.33	
Received from the Treasurer	200.00	
Gift	100.00	
Total Funds available for 1955	$ 346.33	$346.33
Expenses of the Memphis Meeting	$ 15.16	
Expenses of this office, 1955	205.17	
Total Expenses, Fiscal year, 1955	$ 220.33	$220.33
Balance on hand, November 1, 1955		$ 126.00
Expenses, Nov. 1, 1955–Dec. 27, 1955		30.17
Balance on hand, December 27, 1955		$ 95.83

The Secretary's report was accepted and ordered placed on file.

5. *Treasurer* (*R. M. Stabler*): In the absence of the Treasurer, the Secretary read the annual report of that officer. The detailed report was examined, audited, and ordered placed on file. A summary of the report for the fiscal year of November 1, 1954 to October 31, 1955 is as follows:

Balance on hand Nov. 1, 1954	$ 3,515 57
Collections to Nov. 1, 1955	14,908.68
Total funds available for 1955	$ 18,424.25
Total expenditures for 1955	$ 14,007.87
Total cash balance as of Nov. 1, 1955	$ 4,416.38

The expenditures covered costs of Vol. 40 (Nos. 5, 6, and Supplement) of the Journal, cost of Vol. 41 (Nos. 1, 2, 3, and 4)—a total of $12,186.11; AIBS dues of $650.00; expenses of the Editorial Committee, Secretary, and Treasurer amounting to $1,171.76; and travel vouchers of $200.00 as major items.

6. *Custodian of Back Issues* (*W. S. Bailey*): A summary of the major items in the report is as follows:

Credits:		
Balance on hand November 1, 1954	$ 1,452.31	
Accounts collected	1,943.00	
Contributions *	1,450.00	
		$ 4,846.16
Debits:		
Repayment of Chemical Foundation Loan	$ 2,000.00	
Miscellaneous expenses	244.89	
		$ 2,244.89
		$ 2,601.27

The report was audited, accepted and ordered placed on file.

7. *Custodian of the Endowment Fund* (*N. R. Stoll*): The annual report of the Custodian for the fiscal year November 1, 1954 to October 31, 1955, shows a bank balance of $461.33, and a U. S. Savings Bond of January, 1950, carried at its redemption value of $809.00, making a total fund of $1,270.33. Attention of all parasitologists is called to the opportunity of increasing this fund.

* See report of Special Committee on Re-financing of the loan from the Chemical Foundation. Report does not indicate that the Foundation cancelled $500.00 of the original loan of $2,500.00 since no money movement was involved. Of the balance on hand, approximately $2,200.00 is obligated for the reprinting of 14 issues of the Journal now out of stock. The original cost of the Quindex has been amortized and there are still a few copies available for sale.

The report, which had been audited before presentation, was accepted and ordered placed on file with the suggestion that the bank balance might be invested in a manner to bring in a higher rate of interest.

8. *Chairman of the Editorial Committee (G. R. LaRue)*: This report covers six numbers (Vol. 40, No. 6, and Vol. 41, Nos. 1–5, inc.). These six numbers contained 94 research papers, 46 research notes, indices and other matter pertaining to the interests of the Society, totalling 682 pages, at a cost of $10,766.21, amounting to $15.80 per page for editions of 2300 copies. The total cost is, in small part, offset by receipts from advertising and from payments made by authors for excess cost of publishing their papers.

In December, 1954, printing costs exclusive of engraving and paper stock, were advanced 3.5%, and again by the same amount in December, 1955. Engraving costs per unit have remained as before, and paper costs depend upon variations in price set by the mill which supplies the Business Press, our printer. There is reason to believe that the cost of publishing the Journal will continue to increase, perhaps annually.

Some corrected galley proofs for the February issue, 1956, have been turned over to the new Chairman of the Editorial Committee, and there are enough manuscripts on hand and partly processed for about two additional issues. During the past year, publication of most papers has been within eight months after receipt, except for the occasional paper which has required extensive reworking, or has remained overlong on the desk of the author.

In closing this my second and final report, I wish to express my sincere thanks to the members of the Editorial Committee who carried a large share of the editorial burden. I am also grateful to Mr. Everett E. Lund, assisting the Editorial Committee, for certain help, and more particularly for the feeling that he could be called upon when needed. Thanks are also due to the members of the Editorial Board and to others outside the regular editorial groups for their valuable assistance in the reviewing of manuscripts sent them. Their critical comments and suggestions have resulted in marked improvements of the manuscripts submitted. To Mr. George M. Houck, Manager of the Business Press, and his staff are due my grateful thanks for counsel and advice during my term of service.

The report was accepted and ordered placed on file, and a motion of special appreciation for the services of Dr. LaRue and his Committee was ordered placed in the minutes.

II. Reports of Standing Committees

1. *Committee on Visual Education and Instruction (M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder)*: The committee reports that aside from answering numerous requests for information their major activity was in the revision of the list of "Visual Aids to Instruction in Parasitology" that became available last year. This revised mimeographed list should be ready by the spring of 1956 and will be mailed to members of the Society at that time.

The report was accepted and ordered placed on file. The Committee was authorized to continue for another year without change in personnel and a vote of thanks was ordered for the efforts involved in preparing the booklet.

2. *Committee on Nomenclature (A. McIntosh, Chr., G. W. Hunter III, F. J. Kruidenier, G. W. Wharton; N. M. Stoll, Associate)*: No problems were submitted to the committee during the year. The International Committee on Zoological Nomenclature has recognized the existence of the committee and promises to refer problems in the field of Parasitology to it for advice.

The report was accepted and ordered placed on file. The committee, with enlarged responsibilities and re-named "Committee on Nomenclature and Terminology", was continued with no change in personnel for 1956.

3. *Committee on Terminology (H. S. Fuller, Chr., T. W. H. Cameron, G. R. Coatney, E. C. Faust)*: The chairman reported that he had no matters of interest under consideration, and requested that he be relieved of possible future activity in connection with problems of terminology.

The report was accepted and ordered placed on file. In view of the request of the chairman, the committee was discontinued for 1956 and its potential activities transferred to the re-organized Committee on Nomenclature and Terminology.

4. *In Memoriam Committee (E. W. Price, Chr., D. L. Augustine, G. F. Otto)*: The Committee noted the names of six members of our Society who have passed away, five of whom have received recognition in the "In Memoriam" page of the annual Supplement and Program of 1955. In addition they listed the name of James J. Black, an active member since 1927. This information came in too late to be included in the printed list.

The report was accepted with a deep sense of loss to the Society, and ordered placed on file. The committee was continued for 1956 with no change in personnel.

III. Reports of Representatives and Delegates

1. *To Council of the A.A.A.S. (W. Balamuth, G. H. Ball)*: The Representatives presented the minutes of the 1954 meeting of the Council. Most important items dealt with proposed changes in the Constitution of the A.A.A.S. These items were to be voted upon at the 1955 annual meeting of the Council. (Our temporary representatives to the 1955 meetings—E. E. Byrd and G. F. Otto—reported that certain changes were adopted and will be printed at a later date). [See Science, Vol. 123 (3190) : 263–266, 1956].

A copy of the minutes for 1954 and of the agenda for 1955 were ordered placed on file.

2. *To Governing Board of A.I.B.S. (A. C. Walton; L. J. Olivier, alternate)* : Last year our Society voted to support the A.I.B.S. in its move to become an independent entity, separate from the National Research Council. During the year this separation was accomplished and a new Constitution was drawn up, subject to adoption by the various member Societies. At the East Lansing Meeting in September, 1955, members of the Governing Board agreed to present this new Constitution to their several Societies for consideration. Your representatives urge that our Society go on record as favoring the adoption of the new Constitution and in supporting the A.I.B.S. in its renewed attempt to be of major service to the whole field of Biology.

The report was accepted and a copy of the A.I.B.S. Constitution was ordered placed on file. The Council further voted to recommend to the Society that the new Constitution be accepted as printed in the A.I.B.S. Bulletin, and that we retain Society membership for at least one more year even though a slight increase in dues paid from our treasury might result.

3. *To Division of Biology and Agriculture of the National Research Council (A. C. Walton)* : The annual meeting of the Division was held at Washington on May 13 and 14, 1955. Both days were taken up with the reports of the activities of the various committees of the Division during the past year. On the evening of May 13, a panel discussion was devoted to methods of presenting Biology to the Public. Industry, Governmental Agencies, and Science Writers were represented on the panel and each summarized the methods they employed at present and indicated trends for the future. Most of the business of the Division is carried on in committee and the annual meeting is mainly a matter of reports covering these committee activities. Dr. L. A. Maynard was elected chairman of the Division to succeed Dr. Paul Weiss whose term expired in May, 1955.

The report was accepted. Minutes of the meeting are to be printed and will be placed on file when received.

4. *To the Agricultural Research Institute (C. M. Herman)* : Dr. Herman reported on the Fourth Annual Meeting of the Institute, held October 17 and 18, 1955, in Washington. The major purpose of the meetings is to permit representatives of Industry to meet and consult with research workers in the field. Therefore it depends greatly upon the various research groups to suggest types of programs that can integrate the efforts of both Industry and Research.

The report was accepted and ordered placed on file with the suggestion that our representative be empowered to consult with our President as to possible program suggestions to be presented to the A.R.I.

5. *To the International Arid Lands Meetings (C. C. Hoff)* : At the request of the Secretary, Dr. C. C. Hoff of the University of New Mexico served as a delegate to the meetings held April 26 to May 4, 1955, at the University of New Mexico under the auspices of the A.A.A.S. While no direct connection existed between the topics under discussion and the field of Parasitology, it was deemed wise to be represented, and Dr. Hoff served in that capacity.

IV. Reports of Special Committees

1. *Committees to Select Nominees for the Editorial Committee of the Journal of Parasitology:* The reports have already been summarized in the report of the Secretary and the resulting elections to the Committee by votes of Council have been recorded. (A complete file of all correspondence involved is in the Secretary's records).

2. *Committee on "Proposal for Regional Branches of the A.S.P." (D. V. Moore, Chr.)* : The committee recommends that the formation of regional branches be encouraged and that already organized groups be invited to seek affiliation with our Society. The Council agreed that in the proposed revision of the Society Constitution some methods of attaining such ends be incorporated. The Committee further presented certain suggested steps for consideration by the Committee on Constitutional Revision. The committee was discharged with a vote of thanks for its efforts.

3. *Committee on Constitutional Revision (A. O. Foster, Chr.)* : The Committee reported that the problem of revision involved so many ramifications that it has been impossible up to the present to resolve them all or even to consider all of the suggestions received, but major

progress has been made. The committee requested that the study be continud and that the revised document might be ready for the 1956 meeting of the Society. The Council authorized the continuation of the committee without change in personnel. (A. O. Foster, C. G. Huff, M. D. Young, and the Secretary *ex officio*).

4. *Committee on Methods of obtaining Nominations for Offices in the Society* (*E. B. Cram, Chr.*): The committee reported that inasmuch as the matter was also under study by the committee working on revision of the Constitution, it seemed wiser not to suggest changes for the present, but to send suggestions to Dr. Foster's committee for study. The Council approved the suggestion and the committee was discharged with thanks.

5. *Committee on Refinancing of the Chemical Foundation Loan* (*C. G. Huff, Chr.*): The Committee reported that the Chemical Foundation agreed to cancel $500.00 of the original $2,500.00 note on condition that the Society pay off the balance during 1955. Through the generosity of 12 pharmaceutical houses (Abbott Laboratories, American Cyanamid Co., CIBA Pharmaceutical Products Inc., Dow Chemical Co., Dr. Hess and Clark, Dr. Salsbury's Laboratories, Eli Lilly & Co., Pfizer Institute, Pitman-Moore Co., E. R. Squibb & Sons, Sterling-Winthrop Institute, Upjohn Co.) $1450.00 of the amount was donated as a non-recurring gift, and the balance of $550.00 was taken from the funds of the Custodian of Back Issues. The note has been cancelled as paid-in-full, as indicated in the Custodian's report.

The report was accepted and ordered placed on file. A vote of thanks was given to the committee for its successful efforts and a further vote of thanks was extended to the Chemical Foundation and to the Pharmaceutical Houses involved and the Secretary instructed so to notify all parties concerned.

6. *Committee on nomination of papers for consideration of the A.A.A.S. Committee on the Newcomb Cleveland Prize* (*L. A. Stauber, Chr., A. McIntosh, L. O. Nolf*): The committee reported that a selection had been made and reported to the proper A.A.A.S. officials.

The committee was dismissed with thanks for their recommendation.

V. Old Business

1. *Consideration of the Revised Constitution of the A.I.B.S.:* The Council recommended the acceptance of the Revised Constitution and continuance of our Society membership in the A.I.B.S., with instructions to the Secretary to communicate to the proper A.I.B.S. officials the desire of the Society to see more emphasis placed on practical assistance to biologists in general and less emphasis on organizational procedure, now that the A.I.B.S. is free to act independently. It was voted to present this recommendation to the Society for action at the Annual Meeting.

2. *Constitutional Revision:* As indicated under Committee Reports, no final draft of a new or revised Society Constitution is ready for consideration, but the committee still invites suggestions and criticisms from the membership at large.

3. The Secretary reported that in accordance with the directions given last year a contract has been signed with University Microfilms of Ann Arbor, Michigan to put annual Volumes of the Journal of Parasitology on microfilm at the end of each volume. Volume 41 (1955) will be the first volume to be so duplicated. The Society will receive a 10% royalty on each volume sold; these being priced at one-fourth cent per page. No number may be duplicated and sold until the whole volume is completed and in the hands of the regular subscribers.

VI. New Business

1. *Election to Active Membership:* Names of 19 applicants were presented and elected to active membership.

ANDERSON, ROY. C., Dept. Parasitology, Ontario Research Foundation, 43 Queen's Park, Toronto 5, Ontario, Canada.

BURNS, WILLIAM C., Dept. Zoology, University of Wisconsin, Madison 6, Wsconsin.

BUTTREY, BENTON W., Dept. Zoology, University of South Dakota, Vermillion, South Dakota.

COWAN, ARCHIBALD B., Patuxent Research Refuge, Laurel, Maryland.

DUMAS, MARY E., University Station #1, Box 676, Provo, Utah.

DUNCAN, STEWART, Biology Dept., Boston University, 675 Commonwealth Ave., Boston, Massachusetts.

DURRANI, MOHAMMAD Z., 208 Delaware Ave., Ithaca, New York.

EVERHART, BARBARA A., Dept. Veterinary Parasitology, Oklahoma A. & M. School of Veterinary Medicine, Stillwater, Oklahoma.

HUTTON, ROBERT F., State Board of Conservation, Marine Laboratory, Maritime Base, Bayboro Harbor, St. Petersburg, Florida.

LANGE, DARRYL E., Dept. Medical Microbiology, Medical School, University of Wisconsin, Madison 6, Wisconsin.
LYNCH, JOHN E., Pfizer Therapeutic Institute, Maywood, New Jersey.
McLOUGHLIN, DONALD K., Animal Parasite Laboratory, A.D.P., A.R.S., Beltsville, Maryland.
MARGOLIS, LEO, Pacific Biological Station, Nanaimo, British Columbia, Canada.
NESSLINGER, CARLITA L., 960 Fingerboard Road, Staten Island 4, New York.
OLSON, LEROY J., Dept. Bacteriology and Parasitology, Medical Branch, University of Texas, Galveston, Texas.
ROTHENBACHER, HANS J., 105 Gray Hall, University of Arkansas, Fayetteville, Arkansas.
SEO, BYONG S., Room 310, Zoology Building, University of Minnesota, Minneapolis 14, Minnesota.
ST. JOHN, SISTER MARY, Emmanuel College, 400 The Fenway, Boston 15, Massachusetts.
WARREN, McWILSON, Biology Dept., The Rice Institute, Houston, Texas.

2. The Secretary called attention to the fact that there are vacancies in the roster for Life Members and for Honorary Foreign Membership and called for suggested nominations to be considered next year.

3. *Nominations, Elections, and Appointments:*

A. *Nominations:* The Council Committee (E. B. Cram and A. O. Foster) set up to prepare a slate of nominees for offices in the Society reported that only 58 of the 813 ballots sent out were returned with names of suggested candidates for the various offices indicated. In only one case was there a majority agreement on any one name for a particular position. Therefore the Council, acting as a Committee of the Whole, drew up the following slate to be presented to the Society for consideration at the annual business meeting:

President: Eloise B. Cram (was President-Elect during 1955)
President-Elect: Gilbert F. O tto (to succeed E. B. Cram)
Vice-President: Aurel O. Foster (to succeed G. F. Otto)
Secretary: Arthur C. Walton (2-year term to succeed himself)
Council Members at Large: Paul C. Beaver, Francis J. Kruidenier (4-year terms to succeed E. E. Byrd and C. A. Herrick), William Trager (2-year term to replace A. O. Foster, candidate for the office of Vice-President).

B. *Elections:* The Council authorized the following elections to the designated offices: *Editorial Committee:* Leon Jacobs, Chr., Harold M. Manter, Bernard V. Travis (5-year terms to succeed G. R. LaRue, Chr., C. G. Huff, C. B. Philip, resigned); *Editorial Board:* Allen McIntosh, R. Barclay McGhee, Paul P. Weinstein (4-year terms to succeed A. McIntosh, L. J. Olivier, N. R. Stoll).

C. *Appointments:* The Council authorized the following appointments to the designated activities: *Representatives to the Council of the A.A.A.S.:* Raymond M. Cable, Richard P. Hall (2-year terms to succeed W. Balamuth, G. H. Ball).

D. *Committee Appointments:* (Annual appointments in the case of Standing Committees)

a. *Visual Education and Instruction:* M. S. Ferguson, Chr., D. V. Moore, J. C. Swartzwelder (no change in personnel).

b. *Nomenclature and Terminology:* A. McIntosh, Chr., G. W. Hunter III, F. J. Kruidenier, G. W. Wharton. N. R. Stoll, as a member of the I.C.Z.N., was named an Associate of the Committee (no change in personnel, but charged with additional duties relating to Terminology).

c. *In Memoriam Committee:* E. W. Price, Chr., D. L. Augustine, G. F. Otto (no change in personnel).

d. *Special Committee on Constitutional Revision:* A. O. Foster, Chr,. C. G. Huff, M. D. Young. The Secretary is to serve as *ex-officio* member (no change in personnel from 1955).

4. *Annual Meeting:* The Council agreed to present the invitations of the A.A.A.S. (to meet in 1956 in New York during December) and of the A.I.B.S. (to meet in 1956 in Storrs, Connecticut during late August) to the Society for action, but without recommendation.

5. *Other Council Actions:* The Council voted a number of resolutions pertaining to the affairs of the Society.

A. On petition presented by L. A. Stauber, the Council voted to set up a Special Committee on Public Relations to aid in the movement to obtain increased funds for parasitological research and further voted a sum of not over $150.00 to meet necessary expenses of such a committee during 1956. It was further suggested that such a project might well be brought to the attention of the proper A.I.B.S. officials as a future activity on their part. Dr. Otto has been appointed chairman of this Special Committee.

B. On a petition presented by C. G. Huff, the Council voted to set up a Special Committee to collaborate with the NRC committee on Educational Policies, particularly in the evaluation of textbooks in the field of Parasitology. The Special Committee consists of R. D. Manwell, Chr., D. V. Moore, L. R. Penner.

C. On a request from the NRC for financial assistance in printing a supply of their "Life Sciences" booklet to be distributed without cost to Secondary Schools, Council approved the idea but regretted that finances of the Society were such that support of this outside activity was not feasible for the present.

D. A request from F. J. Kruidenier for Society backing in urging the printing of a revision of the Subject Indices on Parasites to parallel the revised Author Indices resulted in the Council authorizing the Secretary to write to the Governmental agencies involved, urging their early attention to this necessary and valuable service to a wide public. Much of the necessary data for such revision are already available in the files of the Department of Agriculture at Beltsville.

E. In connection with the possible printing of a cumulative index for Vols. 26–40 of the Journal of Parasitology, Dr. Bailey was authorized to present the activity for consideration by the Governing Board of the A.I.B.S.

The Council voted to adjourn at 1:40 A.M., December 28, 1955.

Respectfully submitted
A. C. Walton, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS
THIRTIETH ANNUAL GENERAL BUSINESS MEETING
DECEMBER 29, 1955

The general business meeting of the Society was called to order by President C. G. Huff at 1:40 P.M., following the annual luncheon in the Rainbow Roof Room of the Dinkler Plaza Hotel, Atlanta, Georgia. One hundred and forty-one persons were present.

1. Reports of Officers, Custodians, Committees, and Society Representatives were read and approved.

2. The Society had the pleasure of hearing a second progress report by Dr. H. E. Meleney, a Past-President of our organization, based on a committee study of the teaching of Parasitology and Tropical Medicine in Medical Schools. Inasmuch as this report is to be published, no summary will be included here.

3. The names of the 19 individuals elected to Active Membership at the Forty-fifth Council Meeting were read.

4. The names of those designated by vote of Council to hold appointive positions (Officers, Representatives, Committee Members) were read.

5. The slate of nominees as candidates for Society offices and Council Membership, prepared by Council, primarily from the returns of membership balloting, was presented. On motions from the floor, the Secretary was instructed to cast unanimous ballots for the individuals so named to the various positions. The Secretary so voted and election declared in each case.

6. The recommendation of the Council to accept the amended Constitution of the A.I.B.S. and to continue our Society membership in that group was presented and by Society vote was accepted.

7. The invitations of the A.A.A.S. and of the A.I.B.S. to hold our 1956 meetings in connection with these groups were read, and by vote of the Society it was decided to accept the invitation of the A.I.B.S. and to hold the 1956 annual meeting at Storrs, Connecticut, on August 26–30, 1956.

8. The various resolutions concerning the welfare of the Society, as passed by the Forty-fifth meeting of the Council on December 27–28, 1955, were read.

9. President Huff presented a beautiful gavel he had made from African pink ivory wood trimmed in ebony. This gavel is to be the permanent symbol of the presiding officer of the Society. In view of the fact that the new President is to be the first woman to direct the activities of the Society, Dr. Huff presented Dr. Cram with an oversize gavel for personal use in future struggles with a predominantly male organization.

10. Votes of appreciation to the various organizations and individuals concerned with the very successful Thirtieth Meeting of the Society were passed and the Secretary was instructed to convey such appreciation to the proper parties.

11. The Society voted to adjourn at 2:20 P.M.

Respectfully submitted
A. C. Walton, *Secretary*

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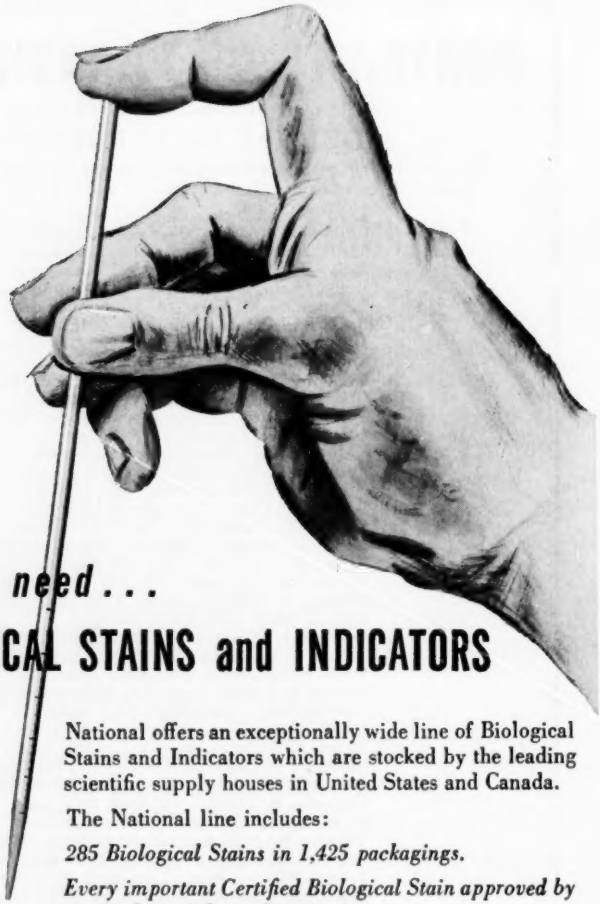

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